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EFFECT OF AUXIN, KINETIN AND GIBBERELLIN ON THE PLACENTAL TISSUE OF *OPUNTIA DILLENII* HAW. CULTURED *IN VITRO*

R. C. SACHAR & R. D. IYER

Department of Botany, University of Delhi, Delhi 8, India

During the course of experiments designed to induce nucellar embryos in *Opuntia dillenii*, we came across an excessive proliferation of the placental tissue which had been excised and cultured along with the ovules. Since this has probably not been described before, we are summarizing our observations below.

It may be mentioned that two previous attempts one by Börger (1926) and the other by Tukey (1937) to grow the excised placenta of some rosaceous fruits proved unsuccessful. In some recent work, carried out in this laboratory on the culture of ovules of *Papaver somniferum*, (Nirmala Maheshwari, 1958), *Argemone mexicana* and *Zephyranthes*, the placental tissue did not respond to any of the nutrient media tried so far.

The ovaries of *Opuntia dillenii* were excised 1-3 days after pollination, flamed after dipping in 90 per cent alcohol, and then cut open into two halves by means of a sterilized scalpel. The placental tissue, with or without the attached ovules, was then scooped out and planted on Nitsch's medium (Nitsch, 1951) supplemented with White's modified vitamin and amino acid solution (White, 1943).[1] This served as the basic medium in all our experiments.

There was no appreciable growth of the placental tissue on the basic medium. An exogenous supply of indoleacetic acid (2 ppm) *or* kinetin (0·5 ppm) also failed to elicit any improvement. However, when kinetin (0·5 ppm) and indoleacetic acid (2 ppm) were supplied together, there was a marked acceleration of growth. The placental fragments showed a conspicuous increase in size due to the addition of new cells resulting from a superficial cambium-like meristem. Evidently, these cell divisions are induced by the synergistic action of kinetin and IAA.

When IAA was replaced by 2, 4-D (2 ppm) in the above medium, the growth of placental tissue was even better. On the surface of the placental mass there arose irregular proliferated mounds of callus tissue (Fig. 1F). Anatomical studies revealed the differentiation of an irregularly oriented meristematic zone which had given rise to the mounds. There was also a differentiation of scattered patches of spiral vascular elements and large pitted cells. Uncultured placentae did not show such elements, but only the normal regular strands.

The placental tissue cultured in the basic medium supplemented with kinetin (0·5 ppm)+IAA (2 ppm)+ gibberellic acid[2] (1 ppm) showed an enormous growth of callus which was capable of continuous subculturing (Fig. 1A-C). The subcultures were maintained in an actively growing state for over seven months by transferring them to fresh media every two weeks. However, it was noticed that the extent of proliferation of the callus mass gradually diminished.

With the increase in the size of the callus, the surface in direct contact with the medium turned reddish-brown and became rigid and corky. The free surface

1. The vitamins and amino acid solution comprised the following: niacin, 1·25 ppm; thiamin, 0·2 ppm; pyridoxine, 0·2 ppm; calcium pantothenate, 0·2 ppm; glycine, 7·5 ppm.

2. The gibberellic acid used here consisted of a mixture of gibberellin A and gibberellic acid (3905-116-B), obtained through the courtesy of Dr F. H. Stodola, U.S.D.A., Peoria, Illinois, U.S.A.

presented a snowy-white appearance owing to the shining papillae, some of which were lobed and filamentous (Fig. 1E). These loosely arranged cells of the callus were easily separable and might prove suitable for single-cell culture (Muir *et al.*, 1954; Nickell, 1956).

There was an accumulation of starch grains in the cells of the callus, although these are absent in the cells of the normal placenta (Fig. 1E). On the other hand, sphaeraphide crystals which are found in abundance in the normal placental tissue were absent in the callus. Another

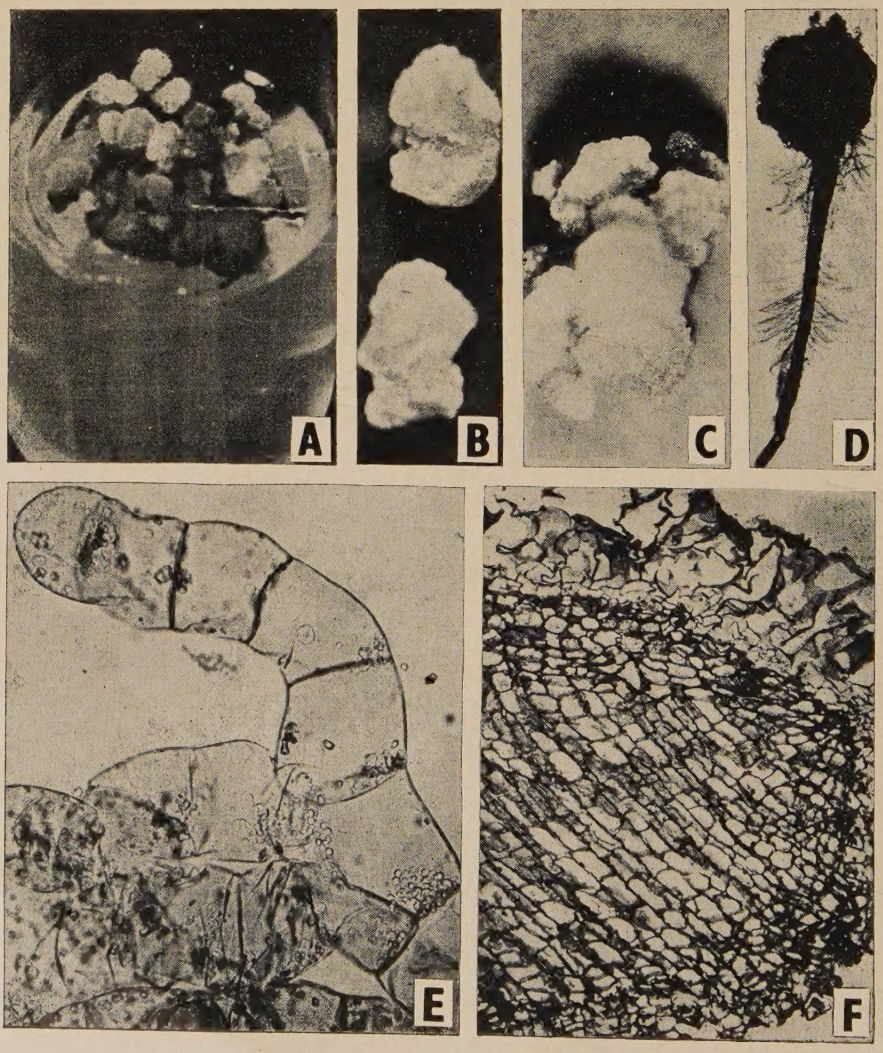

FIG. 1 — Growth of excised placental tissue *in vitro*. A. Five-week old placental callus in basic medium with kinetin 0·5 ppm, IAA 2 ppm and gibberellic acid 1 ppm. × 2. B. Same, five weeks after the first transfer to fresh medium (a liquid medium was tried in this case). × 3. C. Callus, six weeks after third transfer to the same medium as for Fig. A. × 2·5. D. Rooting in callus after four weeks in basic medium containing kinetin 0·5 ppm and adenine 40 ppm. × 9. E. Filamentous row of cells from the surface of the callus shown in Fig. B. × 225. F. Transection of five-week old callus in basic medium with kinetin 0·5 ppm and 2, 4-D 2 ppm. × 110.

difference was that while in the natural placenta the cells become filled with mucilage and the mature seeds are imbedded in a crimson-red tissue, no such change occurred in the cultures.

A placental tissue growing into callus offers promising material for understanding problems of morphogenesis (Wetmore, 1954). Skoog and Miller (1957), working on tobacco pith tissue, obtained a callus which on a kinetin + IAA medium differentiated into root and shoot. Even with the addition of these substances the placental callus of *Opuntia* failed to show such a differentiation, but one of the callus cultures produced a long root with well developed root hairs in a basic medium to which kinetin (0·5 ppm) and adenine (40 ppm) had been added (Fig. 1D). This is a significant deviation from the earlier observation of Skoog and Miller (1957), who found such a combination to be more suitable for shoot formation in tobacco pith callus. Since in *Opuntia* the subculture on adenine + kinetin medium was made from a parent culture containing IAA, kinetin and gibberellic acid, it is quite likely that the callus had already taken up sufficient quantities of auxin to mask the effect of adenine.

As yet, we have only succeeded in culturing placental tissue from pollinated ovaries; it would be interesting to grow placentae excised from very young unpollinated ovaries to see if ovules could be made to differentiate on it.

We are grateful to Professor P. Maheshwari under whose guidance this research was carried out. Thanks are due to Dr B. M. Johri for helpful suggestions.

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MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE FAMILY SANTALACEAE—II. *EXOCARPUS*, WITH A DISCUSSION ON ITS SYSTEMATIC POSITION

MANASI RAM

Department of Botany, University of Delhi, Delhi 8, India

Exocarpus is a root parasite belonging to the family Santalaceae. Of the 17 species, 12 are Australian, the rest being distributed in New Zealand, Norfolk Islands, Malaysia and Madagascar (*see* Ewart, 1930; Pilger, 1935). Due to its apparently primitive floral characters this genus has attracted the attention of many morphologists and has been assigned to various families. Engler & Prantl (1889) and Engler & Diels (1936) placed it in the tribe Anthoboleae as a primitive member of the Santalaceae, while Van Tieghem (1896) raised the above tribe to the rank of a family, the Anthobolacées. Gagnepain & Boureau (1947) proposed a rather drastic change and, on the basis of morphological resemblances with the fossil genus *Sarcopus*, assigned it to the gymnosperms. Lam (1948a) put *Exocarpus* in the group Protangiospermae along with *Salix* and *Casuarina*.

The present study was taken up with a view to determine how far embryological data would support or reject the above assignments. The investigation is based on three Australian species: *E. sparteus* R. Br., *E. cupressiformis* Labill. and *E. strictus* R. Br.

Materials and Methods

The materials were secured from the following sources:

Name of Plant and Place of Collection	Collector	Time of Collection
Exocarpus sparteus		
Applecross, Australia	Dr A. M. Baird	October 1953
E. cupressiformis		
Northbridge, Australia	Prof. H. S. McKee	May 1951
Melbourne, Australia	Miss I. Cookson	August 1951
Victoria, Australia	Miss K. Pike	July 1954
Launceston, Tasmania	Dr T. E. Burns	March 1955
E. strictus		
Rylstone, Australia	Prof. H. S. McKee	August 1951
Launceston, Tasmania	Dr T. E. Burns	March 1955

I take this opportunity to express my indebtedness to all the above-mentioned persons for their generous help.

The mature fruits were treated with 10 to 20 per cent hydrofluoric acid (made up in 70 per cent ethyl alcohol) for 10 to 15 days and run up through the tertiary-butyl-ethyl alcohol series. Sections were cut 5 to 25 microns thick and stained with safranin and fast green.

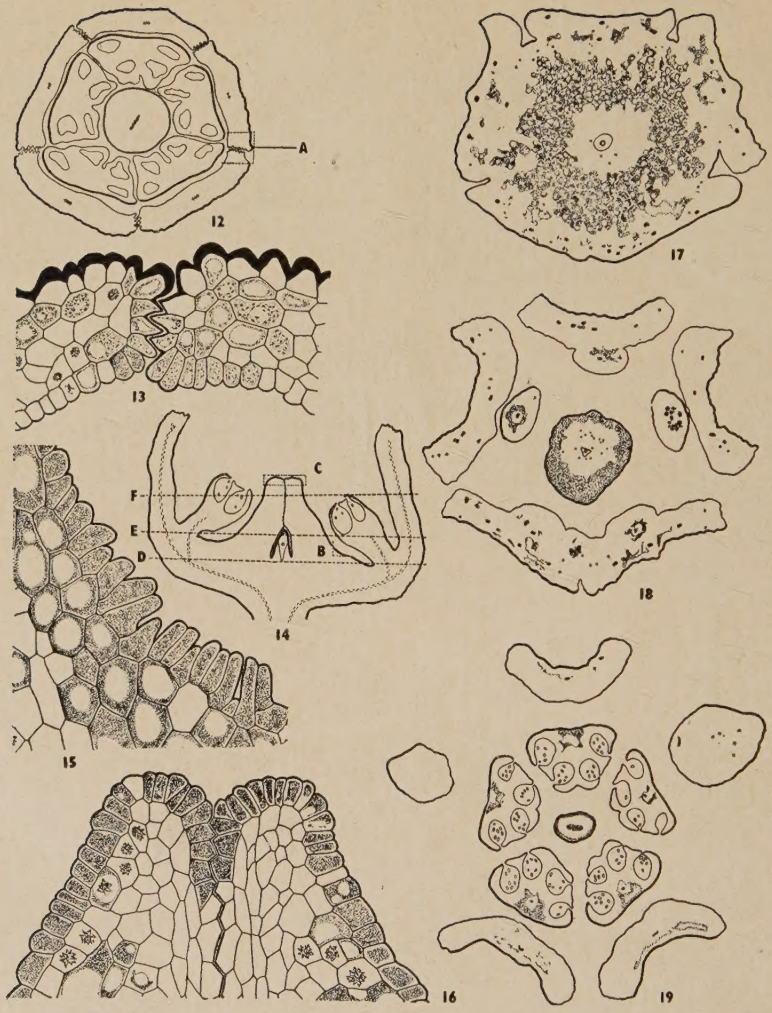
The proembryos with their unusually long suspensors and the endosperms were studied from dissections after softening the fruits in 10 per cent KOH for 12-24 hours.

Since material of the other species was not adequate, the present description is based mainly on *E. sparteus* unless otherwise stated.

Observations

EXTERNAL MORPHOLOGY — The three species can be distinguished from one another by their inflorescences. Two or more racemes are borne at each node in *E. sparteus* (Fig. 1), but only one in *E. strictus* and *E. cupressiformis* (Figs. 4, 9). In *E. strictus* it is rather compressed (Figs. 4, 5). The subsessile, hermaphrodite flowers are arranged spirally on a fleshy axis (Figs. 2, 3, 10) each subtended by a minute bract (Figs. 3, 6, 11). The bract is extremely reduced in *E. cupressiformis* though the vascular supply is recognizable in the vestige. From the inner epidermis of the bract arise numerous thick-walled hairs (Fig. 7) which are very well developed in *E. cupressiformis*.

FIGS. 1-11 — Figs. 1-3. *Exocarpus sparteus*; Figs. 4-8. *E. strictus*; Figs. 9-11. *E. cupressiformis*. (*b*, bract; *h*, hair). Fig. 1. Flowering twig of *E. sparteus* showing two inflorescences at each node. × 2. Fig. 2. Single inflorescence. × 7. Fig. 3. Same, l.s. × 29. Fig. 4. Flowering twig of *E. strictus*. × 2. Fig. 5. Enlarged view of two inflorescences. × 7. Fig. 6. L.s. single inflorescence. × 29. Fig. 7. Portion marked A in Fig. 6. × 217. Fig. 8. T.s. abnormal flower of *E. strictus*. × 50. Fig. 9. Flowering branch of *E. cupressiformis*. × 2. Fig. 10. Single inflorescence. × 7. Fig. 11. Same, l.s. × 29.



FIGS. 12-19 — *E. sparteus*. Fig. 12. T.s. flower. × 57. Fig. 13. Portion marked A in Fig. 12. × 245. Fig. 14. L.s. flower at mature embryo sac stage. × 57. Figs. 15, 16. Portions marked B and C in the preceding figure. × 316. Figs. 17-19. T.s. flower at levels marked D, E and F in Fig. 14. × 57.

The five-partite, persistent perianth lobes are slightly concave at the apices and spread out in a stellate fashion. Occasionally, there may be four or even six segments. The androecium comprises four or five epiphyllous stamens with short and thick filaments and basifixed introrse anthers. The relative disposition of the floral parts is shown in Figs. 14 and 17-19.

The outer epidermis of the perianth segments is covered with a thick cuticle while the inner is filled with tannin (Fig. 13). There are balloon-shaped cells at the apex. In bud condition, the margins of the segments remain united by wedge-shaped epidermal cells which are interlocked with one another (Figs. 12, 13). All the floral organs show an abundance of tannin and sphaeraphides.

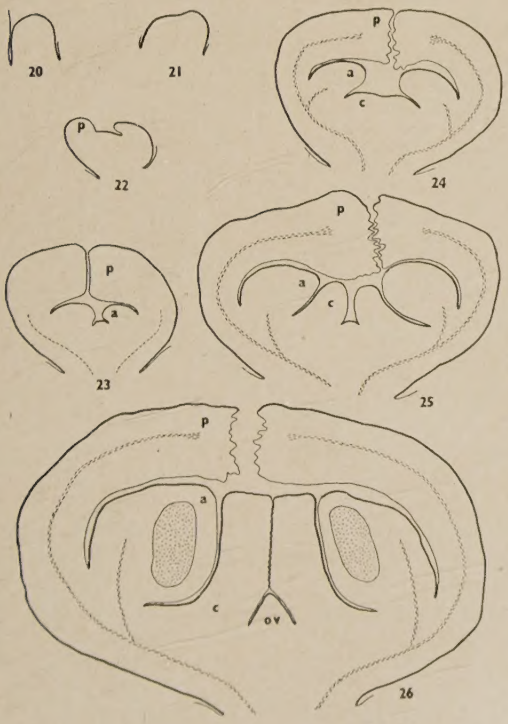
The ovary is semi-inferior and unilocular and contains a single basal ovule. There is a short style and an inconspicuous stigma (Figs. 4, 16) which becomes flared after fertilization. The ovary is surmounted

by a five-lobed disc, each lobe alternating with a perianth segment. In the mature flowers the epidermal cells of the disc elongate and become papillate (Fig. 15).

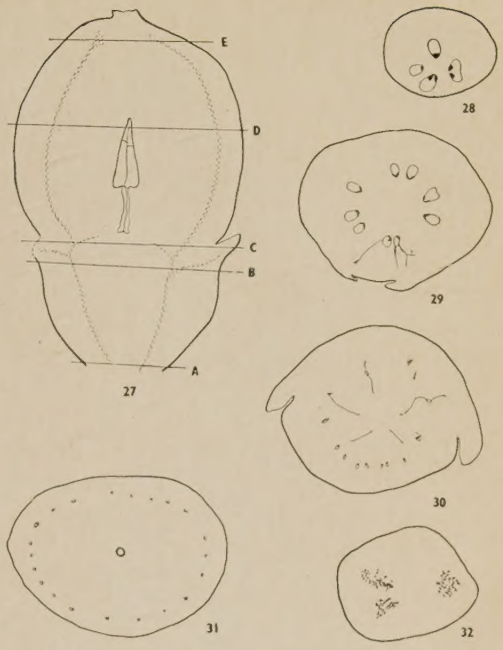
In one flower of *E. strictus* the perianth was five-partite as usual, but there were only four stamens. Three of these occupied their normal position but the fourth was situated between the two perianth lobes (Fig. 8).

ORGANOGENY — The floral primordium makes its appearance as a mass of homogenous cells in the axil of a bract which itself is in an initial stage of development (Figs. 3, 20). The perianth lobes are the first to appear (Figs. 21, 22) followed by one staminal primordium opposite to each perianth lobe (Fig. 23) and then by the gynoecium (Figs. 24-26).

VASCULAR SUPPLY — The vascular bundles are poorly developed even in the mature flowers and their organization was



FIGS. 20-26 — *E. sparteus*. (*a*, androecium; *c*, carpel; *ov*, ovule; *p*, perianth). Organogeny, the dotted portions in Fig. 26 represent the pollen sacs (diagrammatic). × 117.



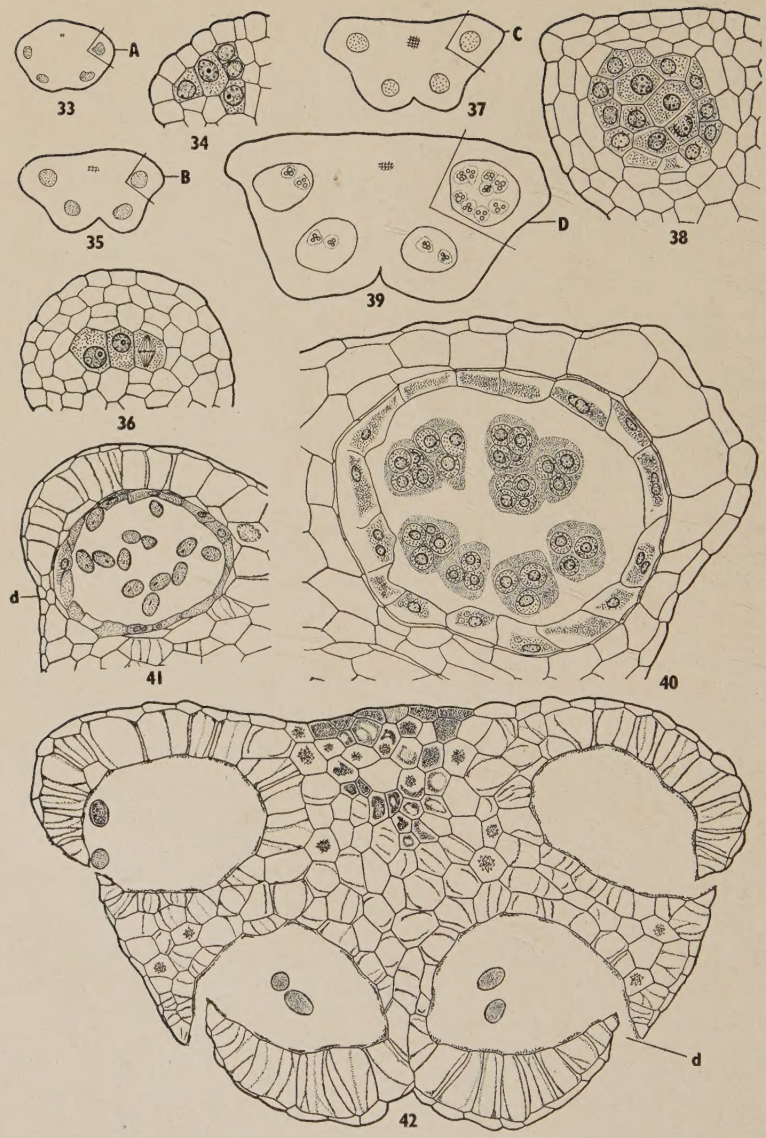
FIGS. 27-32 — *E. sparteus*. Fig. 27. L.s. young fruit (diagrammatic). × 90. Figs. 28-32. T.s. at levels marked A, B, C, D and E respectively. × 24.

studied from young fruits showing a six to eight-celled endosperm. The general plan is represented in Figs. 27-32.

The stele of the inflorescence axis consists of a ring of six bundles and the bract supply arises from one of these. Two bundles enter the pedicel, dividing first into four and then into ten bundles (Figs. 28, 29) which become arranged in a ring. From the five alternating bundles branch off the combined perianth-stamen traces each of which subsequently bifurcates, one branch entering the stamen and the other the perianth lobe (Fig. 29).

At a slightly higher level traces are given off on the inner side (Fig. 30). The bundles of the stelar ring enter the ovary and in its middle region nearly 20 bundles are seen (Fig. 31). Gradually, most of them fade out and only three bundles reach up to the apex of the style (Fig. 32).

MICROSPORANGIUM — Each lobe of the dithecous anther shows a group of hypodermal archesporial cells (Figs. 33, 34). They divide periclinally delimiting the primary parietal layer from the sporogenous



FIGS. 33-42 — *E. sparteus*. (*d*, region of dehiscence). Fig. 33. T.s. young anther. × 134. Fig. 34. Portion marked A in Fig. 33. × 501. Fig. 35. Outline diagram of Fig. 36. × 134. Fig. 36. Enlargement of lobe marked B in Fig. 35. × 501. Figs. 37 and 39. T.s. anthers at mother cell and tetrad stages. × 134. Figs. 38, 40. Portions marked C and D in Figs. 37 and 39 respectively. × 501. Fig. 41. T.s. anther lobe at two-celled pollen grain stage. × 222. Fig. 42. T.s. dehisced anther. × 222.

layer. While the latter undergoes further divisions to form the microspore mother cells, the parietal layer gives rise to the endothecium, an ephemeral middle layer and the glandular tapetum (Figs. 35-38).

At the two-celled stage of the pollen, the epidermis becomes flattened while the cells of the endothecium are radially elongated and show fibrous thickenings. Similar thickenings also appear in the cells of the

partition wall between the pollen sacs. Dehiscence is brought about by the disintegration of two rows of cells on either side of the partition wall. They remain smaller in size and do not develop fibrous thickenings (Figs. 40-42). The middle layer collapses even before the mother cells have undergone reduction divisions. In earlier stages the tapetal cells resemble the microspore mother cells but later they become binucleate and show dense cytoplasmic contents. At about the microspore tetrad stage they become packed with starch grains (Figs. 39, 40). During the enlargement of the pollen grains the tapetal cells become vacuolated and their nuclei fuse. Finally, as the pollen matures the tapetum degenerates.

With the onset of Meiosis I, the protoplasm of the microspore mother cells recedes from the original wall and a special mucilaginous wall is secreted. All the mother cells in a microsporangium do not divide synchronously, and thus while some of them are in prophase I, others will have completed the first meiotic division. In an equatorial view of the metaphase plate, the mother cells showed ten pairs of chromosomes (Fig. 44). The reduction divisions are simultaneous (Figs. 43-49) and the spindles of the homotypic division may be arranged parallel or at right angles to each other. After Meiosis II, the cytoplasm around the daughter nuclei becomes denser and centripetally advancing furrows followed by wedges of the special mucilaginous walls bring about quadripartition (Fig. 50). Both tetrahedral and decussate tetrads have been observed (Figs. 40, 51) but the former are more common. Sometimes one, two, or all the microspores of a tetrad may degenerate. The microspores absorb the special mucilaginous wall, and the original mother wall breaks down liberating the young pollen grains.

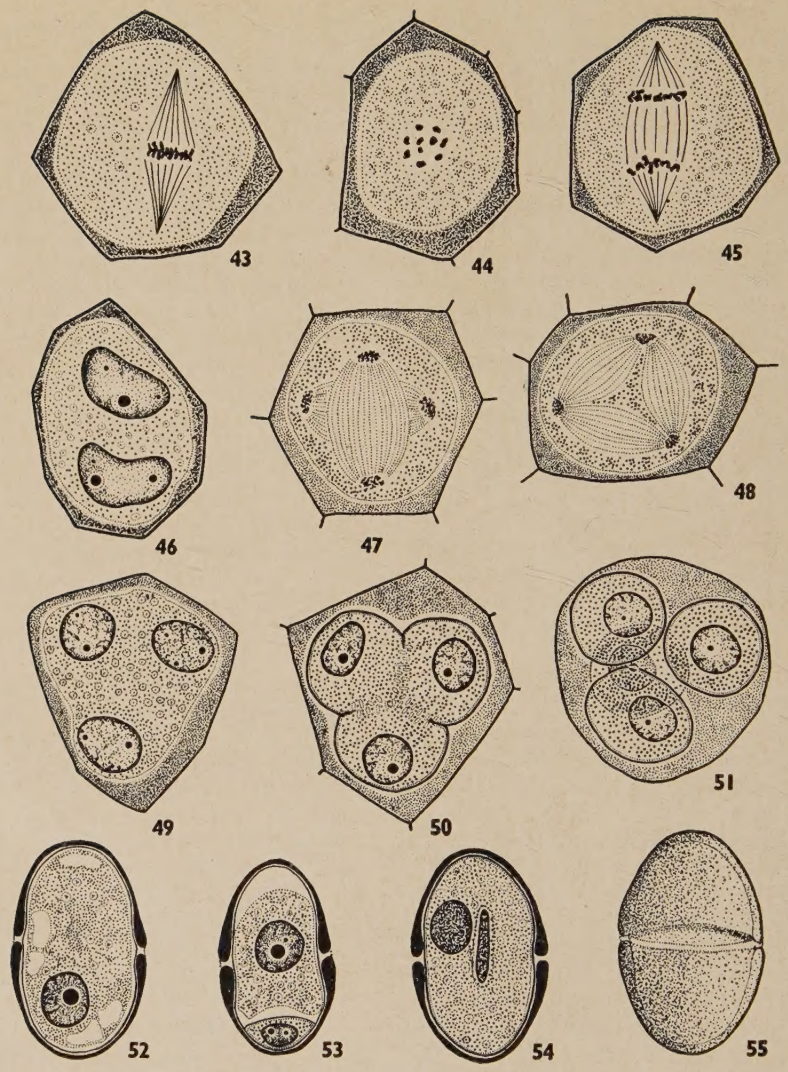
Male Gametophyte — The microspore is densely cytoplasmic. At first the nucleus occupies a central position but due to the appearance of a vacuole it is pushed towards the wall (Fig. 52). Here it divides to form a large vegetative and a small generative cell (Fig. 53). The separating membrane dissolves and the generative cell moves up to the centre of the grain. Its nucleus becomes elongated and is surrounded by a sheath of densely staining cytoplasm (Fig. 54). At this stage the pollen grains accumulate abundant starch grains, and take a deep red stain with safranin which renders them nearly opaque. The mature pollen grains are oblong and the exine does not exhibit any sculpturing. There is a furrow on the equatorial plane with the exine somewhat thicker in the bordering area (Fig. 55). The intine is comparatively thin.

Ovule — The single ovule arises as a conical protuberance from the base of the ovarian cavity. It remains orthotropous and lies in close contact with the ovary wall. The ovule is broad in *E. sparteus* (Fig. 58) but narrow and elongated in *E. cupressiformis* (Fig. 74). There is no clear distinction between the nucellus and the integument. One ovule of *E. sparteus* showed a slight peripheral hump (Fig. 59), which may be said to represent an arrested integument. Periclinal divisions of the epidermal cells were observed in some ovules of *E. strictus* (Figs. 62-64).

Simultaneously with the maturation of the embryo sac, most of the lateral tissue in the upper part of the ovule is absorbed leaving only the epidermal layer intact (Figs. 67, 76). Later on even this is digested. Occasionally, even at the four-nucleate stage (Figs. 66, 75) the upper part of the embryo sac comes to lie in direct contact with the inner epidermis of the ovary (Figs. 61, 68, 77). The antipodal cells disorganize early and thereafter the chalazal end extends towards the base of the ovary.

A feature of unusual interest in the ovule of *E. cupressiformis* is the presence of tracheidal cells which differentiate at the megaspore mother cell stage and show spiral thickenings (Fig. 71). During the development of the female gametophyte a row of tracheidal cells connects the basal end of the embryo sac with the vasculature of the ovary. The tracheidal cells remain recognizable until the endosperm becomes 10 to 16-celled (Fig. 88).

Megasporogenesis and Female Gametophyte — A single hypodermal archesporial cell differentiates in the nucellus and functions directly as the megaspore mother cell (Figs. 56, 62, 70, 71). Of the two dyad cells formed as a result of



FIGS. 43-55 — *E. sparteus*. Figs. 43-49. Microspore mother cells at Meiosis I and II. × 1556. Fig. 50. Cytokinesis by furrowing. × 1556. Fig. 51. Tetrahedral tetrad. × 1556. Figs. 52-54. One and two-celled pollen grains. × 1556. Fig. 55. Surface view of mature pollen grain. × 1556.

Meiosis I (Fig. 72), the upper is invariably the smaller (Fig. 73). Linear and T-shaped tetrads are common in *E. strictus* (Fig. 63), but in *E. sparteus* 'triads'* are more frequent (Fig. 57) and tetrads are rarer (Fig. 58). Only ' triads ' have been observed in *E. cupressiformis* (Fig. 74).

*After the first meiotic division only the lower dyad cell divides while the upper remains undivided.

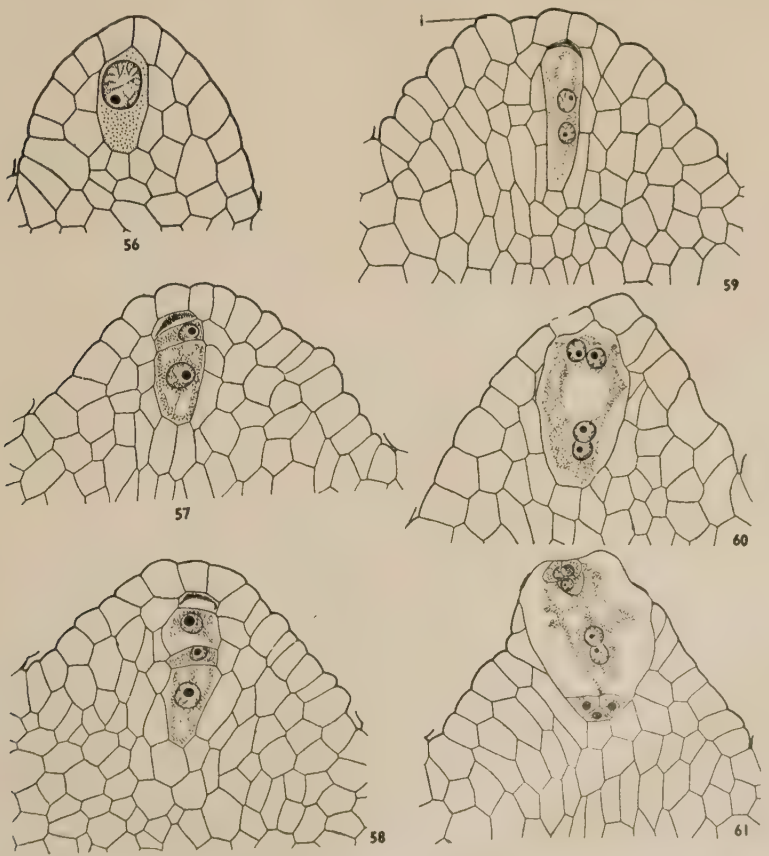
Generally the chalazal megaspore functions and shows a centrally situated nucleus with a vacuole on either side (Figs. 64, 74). In one case the lower two megaspores had enlarged while the upper two had degenerated (Fig. 63).

The two, four and eight-nucleate gametophytes are formed in the usual way (Figs. 59-61, 65-67, 75, 76). In *E. sparteus*, the antipodal nuclei often de-

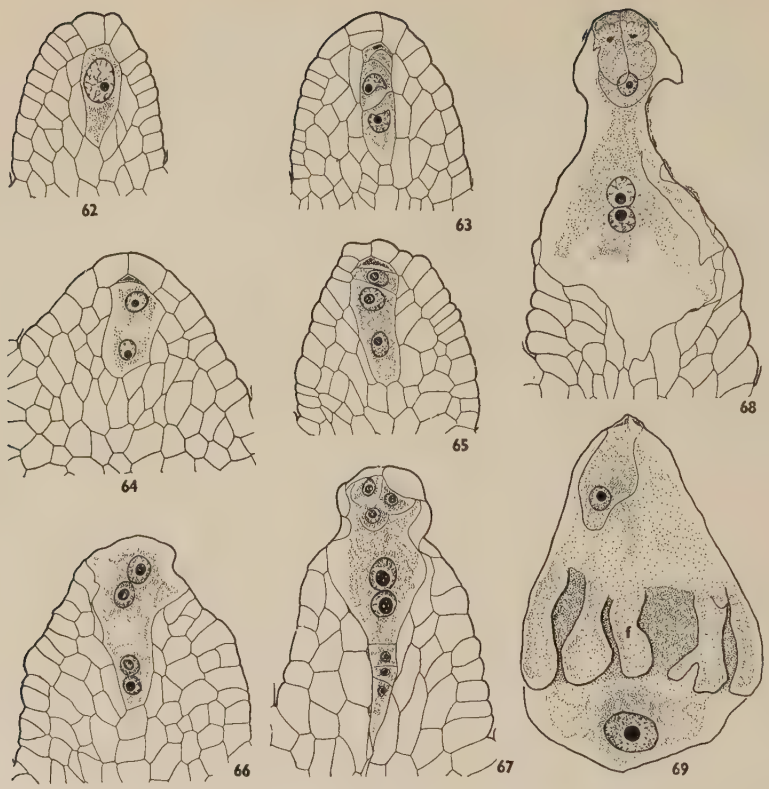
generate even before the organization of the embryo sac. The mature gametophyte of *E. sparteus* is more or less ovoid (Fig. 61) while that of *E. cupressiformis* is longer than broad (Fig. 77). In both these species the synergids are vesicular with a basally situated nucleus simulating the egg (Figs. 61, 76). However, in *E. strictus*, the synergids are large, elongated and hooked with a distinct filiform apparatus (Fig. 68). The polar nuclei fuse in the centre of the embryo sac and the fusion nucleus moves down to the chalazal end. It is usually surrounded by dense cytoplasm and starch grains (Figs. 69, 77).

In *E. strictus* numerous finger-like outgrowths arise from the middle of the embryo sac and hang over the lower portion like a skirt (Fig. 69). Their exact role could not be ascertained but probably they aid in providing an additional absorptive surface.

ENDOSPERM — The primary endosperm nucleus divides much earlier than the zygote. The first division is followed by a transverse wall partitioning the embryo sac into two chambers (Figs. 78, 79). The micropylar chamber divides transversely while the chalazal undergoes a longitudinal division (Figs. 80, 81). The two lower cells do not divide any further but elongate enormously penetrating into the base of the ovary and functioning as haustoria. During elongation, they become separated from each other, grow independently and develop numerous finger-



FIGS. 56-61 — *E. sparteus*. (*i*, ' integument '). Fig. 56. L.s. ovule showing megaspore mother cell. × 593. Fig. 57. Same; with a row of three cells, the uppermost degenerated cell probably represents the dyad while the lower two are megaspores. × 593. Fig. 58. Same, linear tetrad of megaspores. × 593. Figs. 59, 60. Two and four-nucleate embryo sacs. × 593. Fig. 61. Mature embryo sac; the tip of the ovule has been consumed by the gametophyte. × 593.



FIGS. 62-69 — *E. strictus* (*f*, finger-like processes). Fig. 62. L.s. ovule at megaspore mother cell stage. × 447. Fig. 63. Linear tetrad of megaspores. × 447. Figs. 64-66. Two and four-nucleate embryo sacs. × 447. Fig. 67. Organized 8-nucleate gametophyte. × 447. Fig. 68. Same, advanced stage; the antipodals have degenerated. × 447. Fig. 69. Fertilized embryo sac (whole mount); the middle portion has developed a fringe of finger-like processes. × 252.

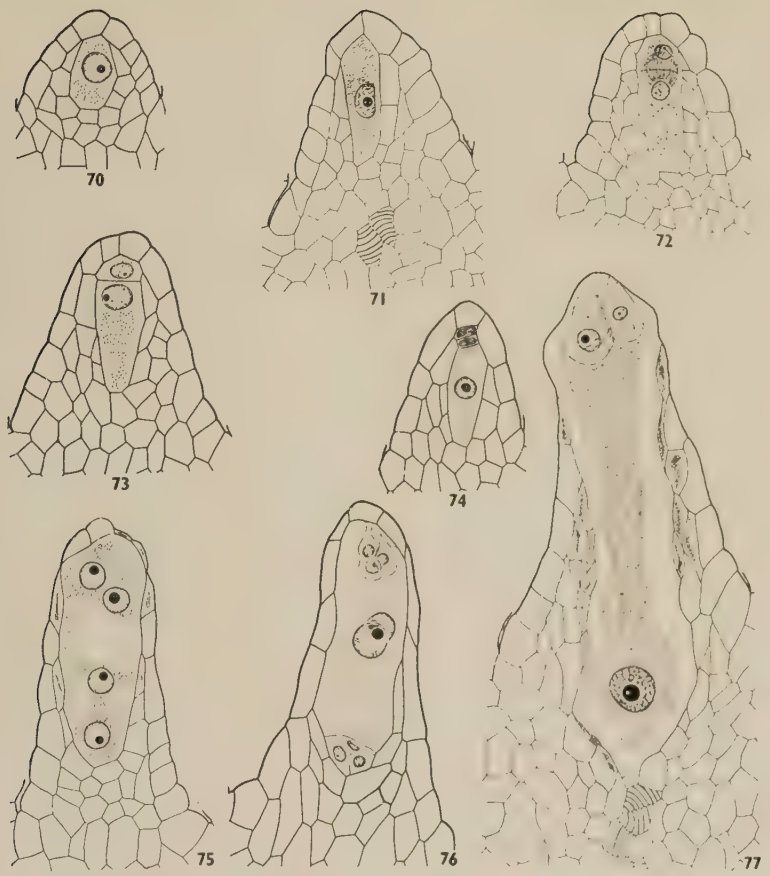
like processes at the basal end (Figs. 82, 83). The haustoria with their hypertrophied nuclei can be traced as late as the dicotyledonous stage of the embryo.

The two micropylar cells divide repeatedly and give rise to the endosperm proper. The apical cells are fairly large and vacuolated and ultimately disorganize (Figs. 84-86). The remaining cells are smaller, have denser cytoplasm, and continue to divide leading to the development of a massive endosperm tissue (Figs. 80-86).

Due to the aggressive activity of the endosperm, the parenchymatous endocarp is gradually consumed so that only its degenerated remains persist as a thin layer outside the ruminate endosperm.

The outer tangential walls belonging to the epidermis of the endosperm develop a thick cuticle (Fig. 123) and the cells around the embryo get depleted of their contents.

In *E. cupressiformis* the micropylar chamber is larger than the chalazal (Fig. 87), but as in the other species it divides repeatedly and produces a mass of endosperm cells (Figs. 87-89, 92, 94, 96). The chalazal chamber elongates and develops two finger-like processes at the basal end (Fig. 89). The available material did not show the earlier stages of cell formation in the haustorium. At the peak of its growth the haustorium becomes a massive cellular structure (Figs. 90-93). Subsequently the cells lose their contents and collapse (Figs. 94-96) but their remnants can be recognized for a long time.



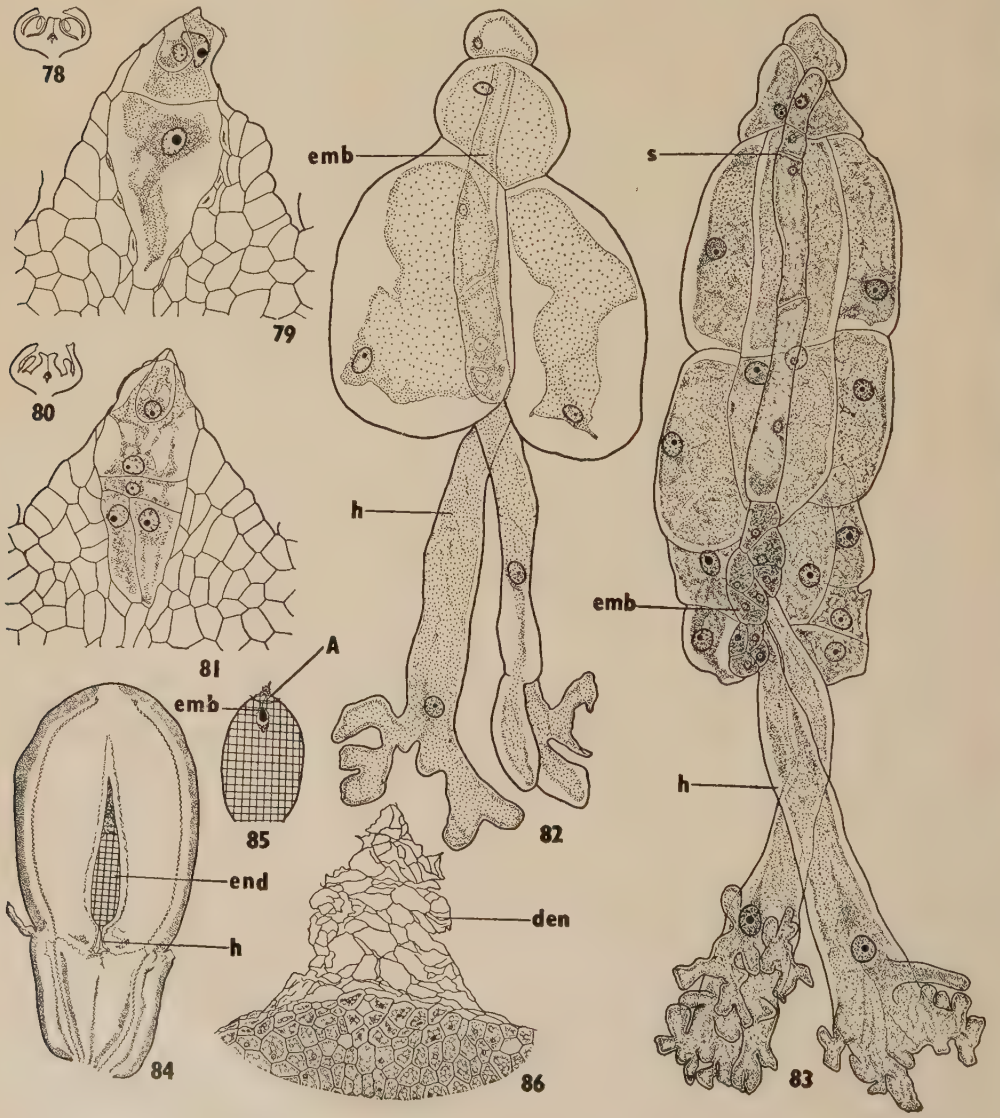
FIGS. 70-77 — *E. cupressiformis*. Figs. 70, 71. L.s. ovules showing hypodermal archesporial cell and megaspore mother cell. × 486. Figs. 72, 73. Division of megaspore mother cell and dyad. × 486. Fig. 74. Triad; the uppermost degenerated cell probably represents the undivided dyad cell. × 486. Fig. 75. Four-nucleate embryo sac. × 486. Fig. 76. Organized 8-nucleate gametophyte. × 486. Fig. 77. Mature embryo sac; the tip has grown beyond the ovule while the chalazal end abuts against the ' tracheidal ' cells. × 486.

EMBRYOGENY — When the endosperm has reached the 8 to 10-celled stage, the zygote undergoes a downward elongation and divides transversely (Fig. 97). Both the daughter cells continue to divide transversely forming a uniseriate filamentous proembryo (Figs. 98-100). Only once I saw a longitudinal division of the terminal cell (Fig. 101). Later divisions are irregular and progressive stages in development are represented in Figs. 102-106. It is to be noted that young proembryos are not easy to trace in microtome sections because of their long and slender nature. They can be more easily studied by dissection.

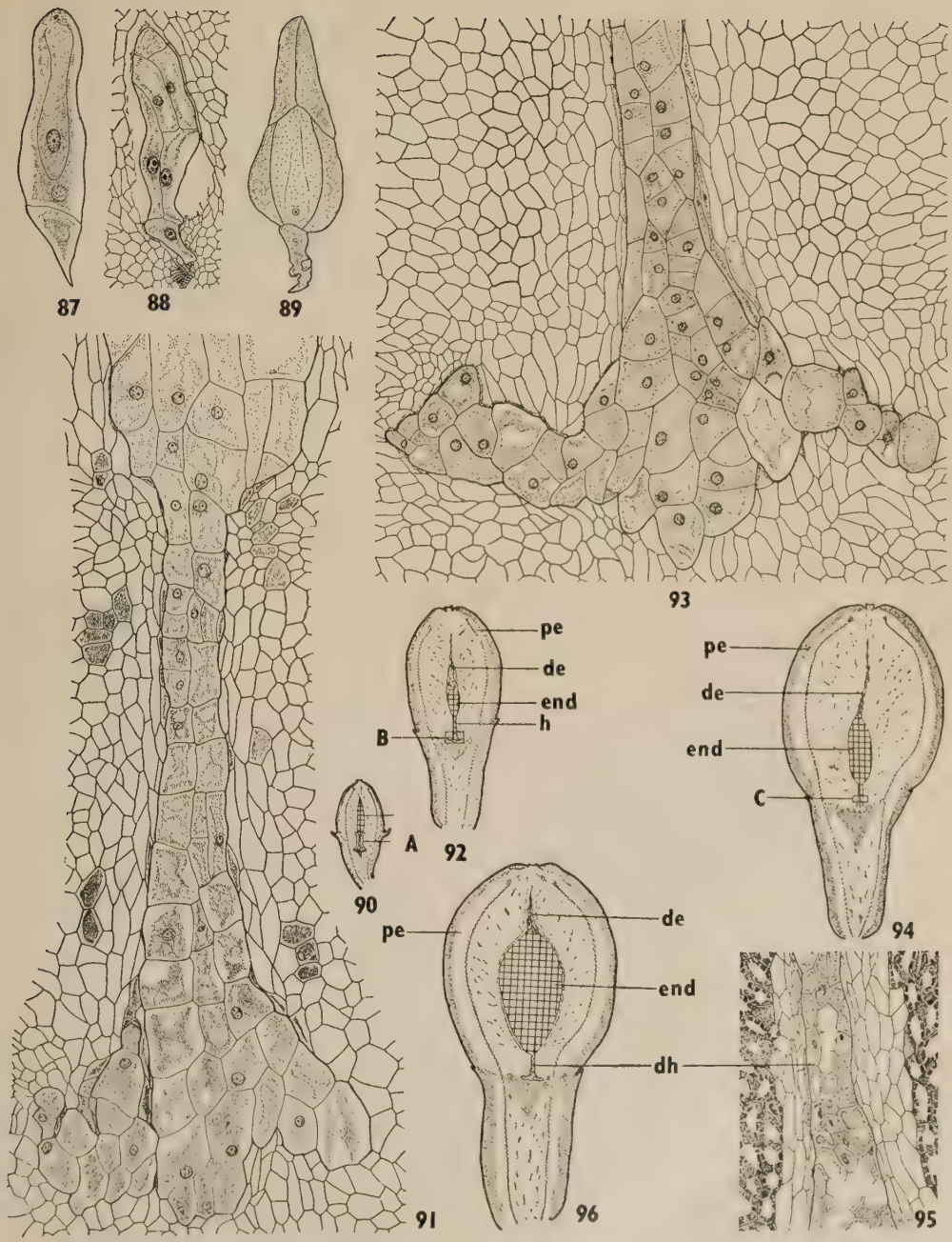
Due to irregular divisions, at places the suspensor becomes thick (Figs. 103-106). Whereas the cells in the upper portion of the proembryo are conspicuously elongated and vacuolated, those at the tip are smaller and richly cytoplasmic. The apical cells divide anticlinally and periclinally leading to the formation of the globular and heart-shaped stages (Figs. 107, 108), and finally a dicotyledonous embryo (Fig. 109).

POLYEMBRYONY — An interesting feature in *E. sparteus* is the development of additional proembryos by the proliferation of suspensor cells (Figs. 110-116). Fig. 110 shows a five-celled proembryo in

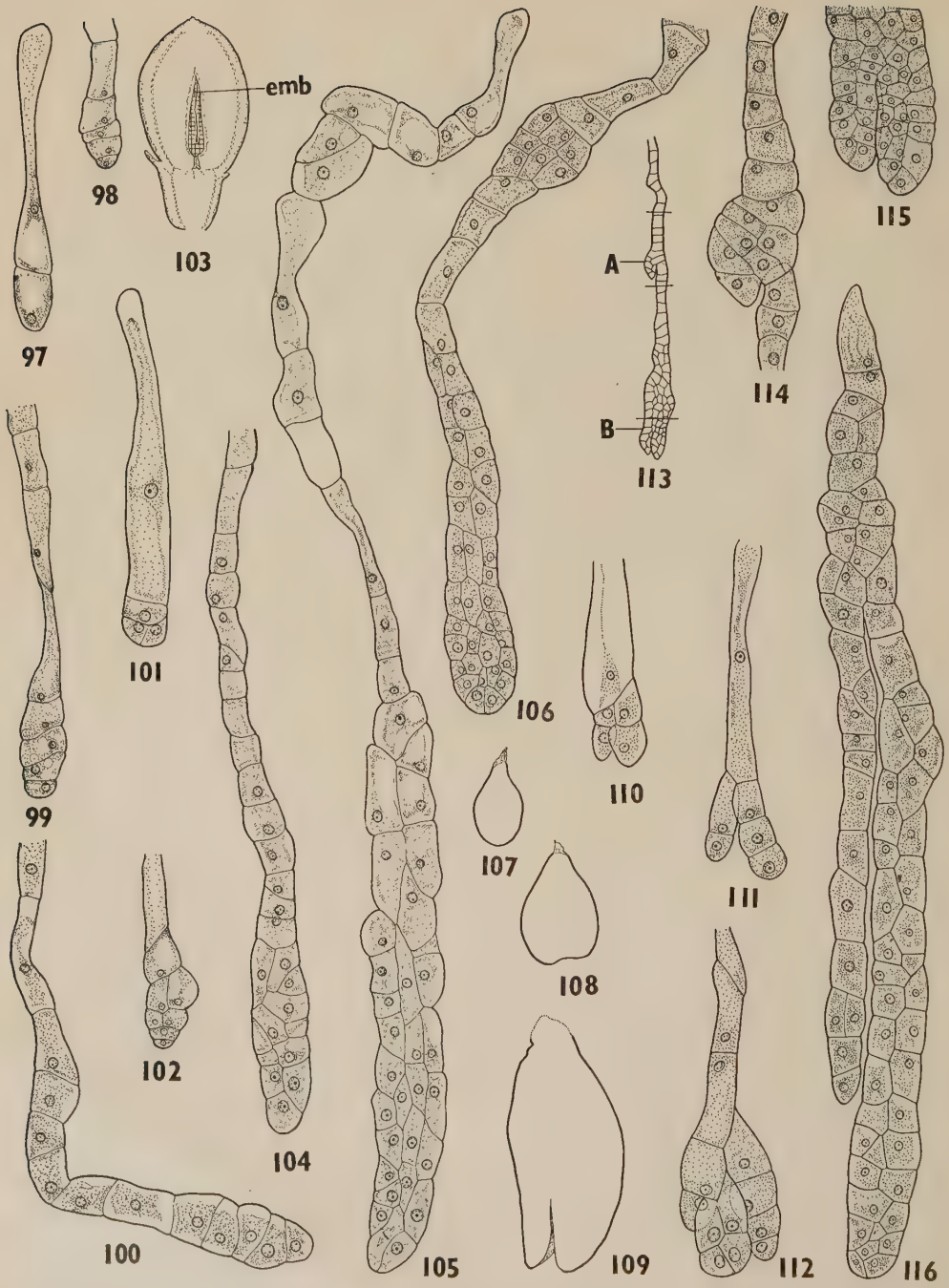
which the two terminal cells have started enlarging independently. Another embryo showed an additional two-celled and a three-celled proembryo attached to a common suspensor cell (Fig. 111). Proliferation may not be confined to the terminal cells for intermediary cells are also often involved (Figs. 114, 116). Even though several proembryos develop concurrently for some time, one of them ultimately takes the lead (Fig. 109). None of the fruits ever showed more than one embryo.



FIGS. 78-86 — *E. sparteus*. (*den*, degenerated endosperm; *end*, endosperm; *emb*, embryo; *h*, haustorium; *s*, suspensor). Figs. 78, 80. Outline diagrams of Figs. 79 and 81 respectively. × 12. Figs. 79, 81. Ovules enlarged to show two and four-celled endosperm. × 434. Figs. 82, 83. Whole mounts of endosperms with proembryos; note the irregularly lobed bases of the chalazal haustoria. × 434. Fig. 84. L.s. fruit at a later stage of endosperm development (diagrammatic). × 12. Fig. 85. L.s. endosperm at the globular stage of proembryo (diagrammatic). × 12. Fig. 86. Enlarged view of portion marked A in Fig. 85. × 116.



FIGS. 87-96 - *E. cupressiformis* (*de*, degenerated endosperm; *dh*, degenerated haustorium; *end*, endosperm; *h*, haustorium; *pe*, pericarp). Fig. 87. Two-celled endosperm with elongated zygote (whole mount). × 200. Figs. 88, 89. Young endosperms. × 200. Figs. 90, 92, 94. L.s. fruits at different stages of endosperm development (diagrammatic). × 7. Figs. 91, 93, 95. Portions marked A, B and C respectively in Figs. 90, 92 and 94. × 200. Fig. 96. L.s. fruit showing advanced stage of endosperm (diagrammatic). × 7.



FIGS. 97-116 — *E. sparteus* (*emb*, embryo). Fig. 97. Two-celled proembryo. × 226. Figs. 98-102, 105. Stages showing progressive development of proembryo. × 226. Fig. 103. L.s. fruit showing endosperm and embryo (diagrammatic). × 8. Fig. 104. Enlarged view of embryo from Fig. 103. × 226. Figs. 106-108. Globular and heart-shaped embryos. × 52. Fig. 109. Dicotyledonous embryo (diagrammatic). × 52. Figs. 110-113, 116. Polyembryony. × 226. Figs. 114, 115. Portions marked A and B in Fig. 113. × 226.

PERICARP — At the time of fertilization the ovary wall consists of eight to ten layers of cells (Figs. 117, 118). Two to three hypodermal layers contain tannin and are followed by a zone of parenchymatous cells. Sphaeraphides are common in the latter. Due to divisions in the inner hypodermal region, the pericarp increases considerably in bulk (Figs. 119, 120), and finally differentiates into three zones — epicarp, mesocarp and endocarp. The epicarp comprises the outer thickly cutinized epidermis with six to eight layers of tannin-filled cells which loosen at maturity. The cells of the mesocarp become sclerotic and their walls show numerous pit canals; its outermost layer also contains crystals (Fig. 122). Next is the vascular region. The endocarp remains parenchymatous and is consumed by the endosperm.

While the above changes are taking place in the ovary wall, the cells of the pedicel multiply and enlarge considerably forming a bulbous structure at the base of the fruit.

The fruit is a small, globular, ovoid nut raised on a swollen peduncle. The seed is ' naked ' in the sense that it lacks a testa. The small embryo with its short cotyledons occupies an apical position in the massive endosperm (Figs. 121, 124, 125).

Systematic Position

Gagnepain & Boureau (1947) isolated the genus *Exocarpus* from the Santalaceae, assigned it to a unigeneric family and classified it under the gymnosperms on the basis of the following characters: (1) The cladodes are similar to those of *Phyllocladus*, (2) the fleshy pedicel is like that of *Acmopyle* and *Podocarpus*, (3) the ovule is naked and has a pollen chamber, and (4) the fruits resemble those of the Taxaceae.

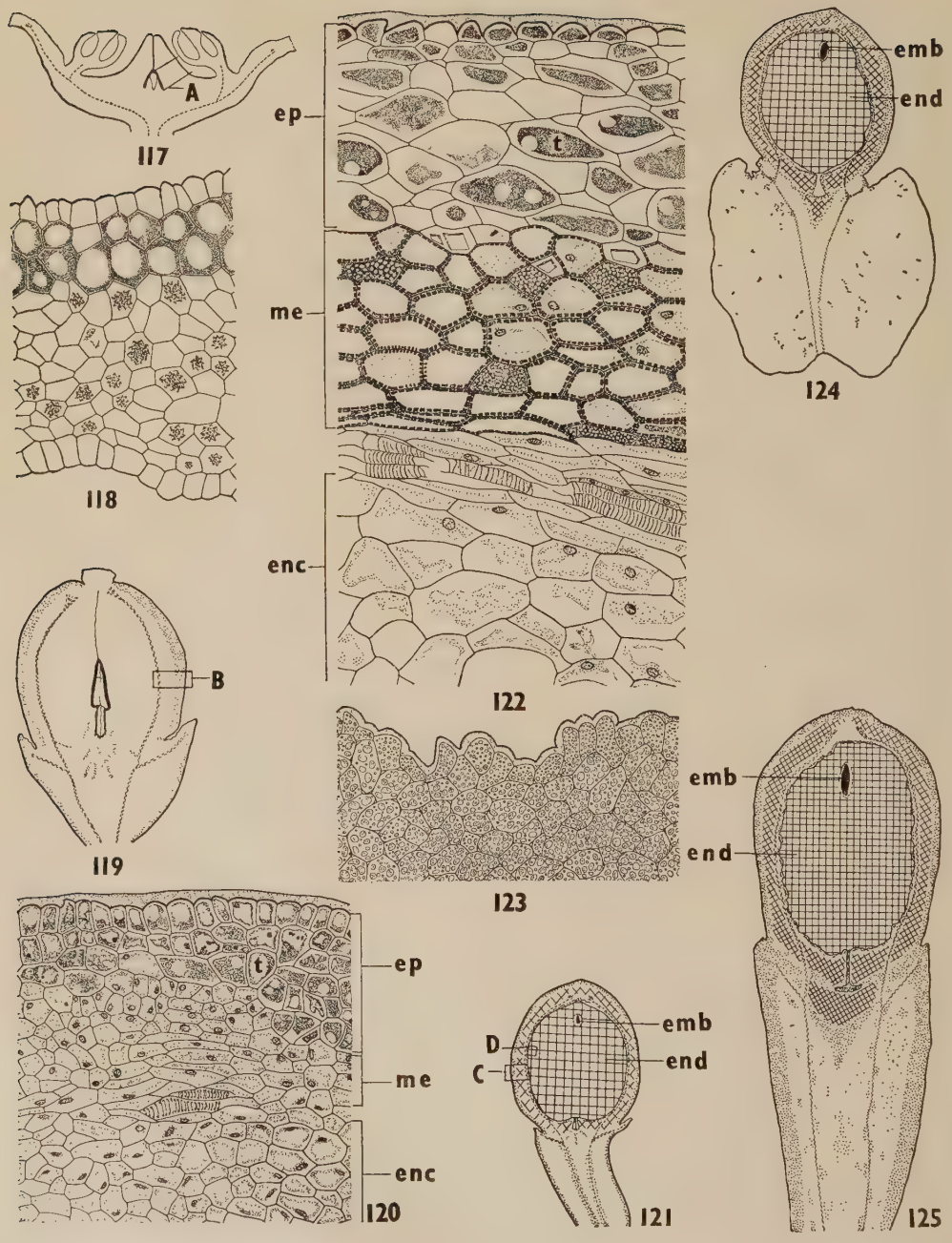
The simple structure of the ovule led Lam (1948a, b) to consider it to be a protangiosperm.

Bailey (1949) and Eames (1950) have criticized the conclusions of Gagnepain & Boureau (1947) and Lam (1948a, b). Bailey writes: " If the Exocarpaceae are to be removed from the Santalaceae and placed in closer relationship with the gymnosperms, such a transfer should be based, not solely upon superficial examinations of the flower of a single species, but upon, comprehensive cytological, embryological, anatomical and general morphological studies of *Exocarpus* in comparison with other genera of the Santalaceae . . .". Süssenguth (1954) also wrote: " Da der Embryosack von *Exocarpus* bisher nicht bekannt ist, ist nach Ansicht des Ref. eine endgültige Beurteilung der Frage noch nicht möglich, wenn schon andere starke Übereinstimmungen von *Exocarpus* mit wirklichen Santalaceen sowie die eindeutige Angiospermie der Eu- Santalaceae zunächst gegen die Annahme von Gagnepain und Boureau sprechen. Soviel dürfte schon jetzt feststehen, das die Herausnahme von *Exocarpus* (und eventuell verwandter Gattungen) nur zu halten wäre, wenn mann gleichzeitig eine Annäherung der übrigen Santalaceae (und Loranthaceae) an die Gymnospermae stützen könnte."

We may now examine how far embryological data can help in the solution of this problem. The ovule of *Exocarpus* shows a single hypodermal archesporial cell which directly functions as the megaspore mother cell; the embryo sac is of the Polygonum type (it does not extend into the style but elongates downwards up to the base of the ovary); the endosperm is Cellular with a characteristic chalazal haustorium; the first division of the zygote is transverse; the proembryo has a long, well-developed suspensor and the fruit wall consists of the parenchymatous epicarp and the stony mesocarp.

Thus, there is not the slightest similarity between *Exocarpus* and gymnosperms. The floral anatomy of the pistillate flowers of *Exocarpus* is somewhat similar to those of *Santalum* and the staminate flowers resemble *Leptomeria* and *Choretrum* (Smith & Smith, 1942). Moreover, the wood anatomy of *Exocarpus* also closely resembles that of *Santalum*, *Osyris*, *Fusanus*, etc. (Swamy, 1949; Metcalfe & Chalk, 1950). Pollen grains similar to those of *Exocarpus* are also found in *Omphacomeria* — another genus of the Santalaceae.

It is thus concluded that *Exocarpus* is a confirmed angiosperm correctly assigned to the family Santalaceae. Althoug this



FIGS. 117-125 — Figs. 117-123. *E. sparteus*; Fig. 124. *E. strictus*; Fig. 125. *E. cupressiformis*. (*enc*, endocarp; *end*, endosperm; *emb*, embryo; *ep*, epicarp; *me*, mesocarp; *t*, tannin). Fig. 117. L.s. flower at mature embryo sac stage (diagrammatic). × 9. Fig. 118. Enlargement of portion marked A in Fig. 117. × 254. Figs. 119, 121. L.s. fruits (diagrammatic). × 9. Figs. 120, 122. Portions marked B and C in Figs. 119 and 121. × 254. Fig. 123. Endosperm cells from region D marked in Fig. 121. × 254. Figs. 124, 125. Mature fruits, l.s. (diagrammatic). × 7.

genus exhibits considerable reduction in its vegetative and reproductive organs, and the embryological features are not fully identical with other members of the Santalaceae, the differences are not sufficiently distinctive to justify the removal of *Exocarpus* to a separate family or order.

Summary

The floral morphology and embryology of three species of *Exocarpus* (*E. sparteus*, *E. strictus* and *E. cupressiformis*) have been studied.

The inflorescence is a raceme borne singly or in pairs at each node. The flowers are sessile and pentamerous but tetra- or hexamerous conditions may also occur. The stamens are opposite and isomeric with the perianth lobes.

The ovary is semi-inferior and unilocular with a single, basal and sessile ovule.

The anther wall comprises the epidermis, fibrous endothecium, a single ephemeral middle layer and the glandular tapetum. The anther dehisces by four longitudinal slits.

The reduction divisions are simultaneous producing tetrahedral or decussate tetrads. The mature pollen grains are oblong with a median germinal furrow, and are shed at the 2-celled stage.

The ovule does not show any sharp distinction into a nucellus and the integument.

A single hypodermal archesporial cell differentiates in the nucellus and functions directly as the megaspore mother cell. In *E. cupressiformis* only ' triads ' have been observed, but in the other two species tetrads are of common occurrence. The development of the embryo sac conforms to the Polygonum type. The antipodal cells degenerate early.

Due to the absorption of the apical cells of the ovule, the tip of the embryo sac becomes exposed; its chalazal end elongates downwards to the base of the ovary.

The endosperm is Cellular. There is a 2-celled chalazal haustorium in *E. sparteus* and a multicelled haustorium in *E. cupressiformis*.

The first division of the zygote is transverse. Further transverse divisions give rise to a long filamentous embryo. There are indications of polyembryony due to the proliferation of the suspensor cells. However, ultimately only a single embryo reaches maturity.

Due to the aggressive activity of the endosperm proper, the ovular tissue and parenchymatous endocarp are used up so that the seed becomes ' naked '. In a ripe fruit the pericarp consists of only the stony mesocarp and the tanniniferous epicarp.

The embryological data clearly indicate that *Exocarpus* is an undoubted angiosperm and is a reduced member of the family Santalaceae.

I am greatly indebted to Dr B. M. Johri and Professor P. Maheshwari for suggesting this problem and for their valuable guidance in the preparation of this paper.

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MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE FAMILY SANTALACEAE—III. *LEPTOMERIA* R. BR.

MANASI RAM

Department of Botany, University of Delhi, Delhi 8, India

The genus *Leptomeria* contains 15-16 species distributed in western and southern Australia and Tasmania. The earlier work on the embryology of the Santalaceae has already been referred to (Ram, 1957). So far there has been no work on *Leptomeria*. The following account deals with the morphology and embryology of *L. cunninghamii* Miq. and *L. acida* R. Br.

The material was secured from Australia through the courtesy of Professor H. S. McKee and Dr A. M. Baird. It was dehydrated in the tertiary-butyl-ethyl alcohol series and prepared for microtomy in the usual way. The tortuous embryo sacs and earlier stages of development of endosperm were also studied from dissections.

Observations

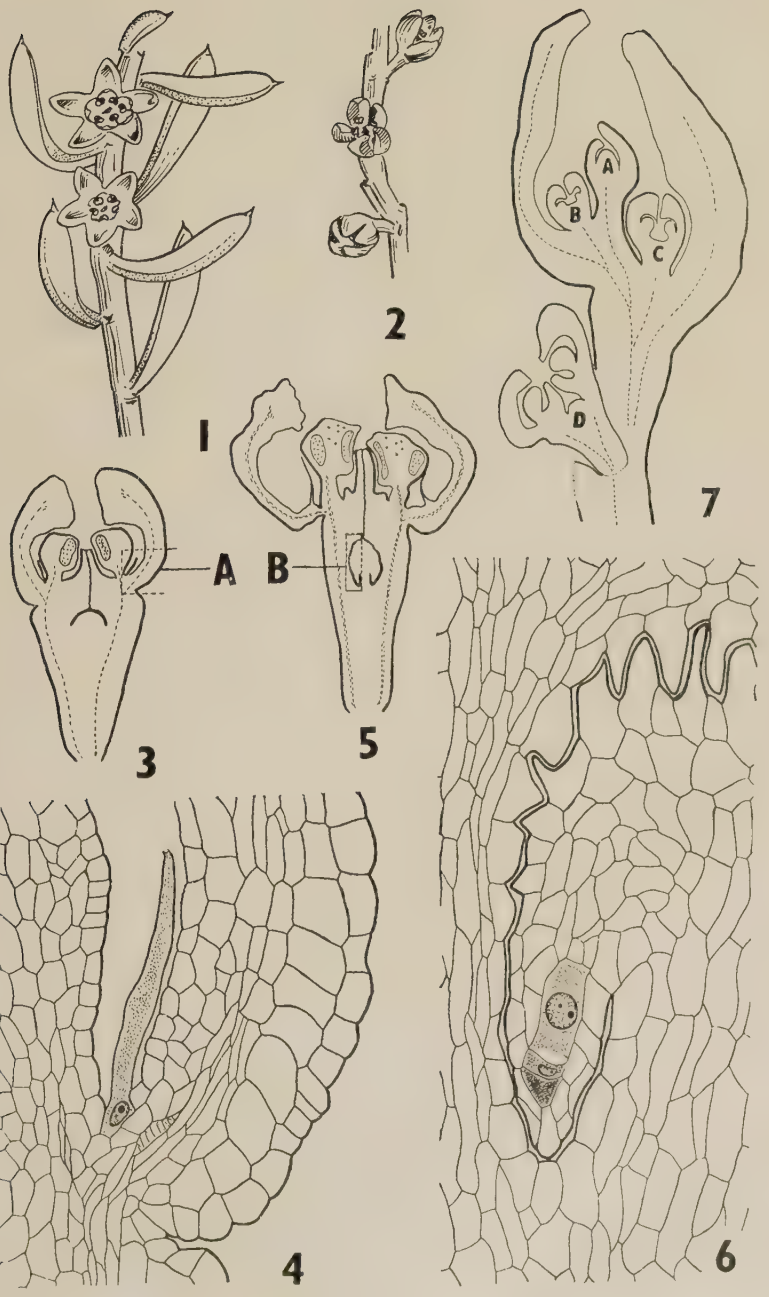
Floral Morphology — In both the species the inflorescence is a raceme. The flowers are pentamerous, actinomorphic and hermaphrodite, and each flower is borne in the axil of a bract. The latter is leafy and persistent in *L. cunninghamii* (Fig. 1), but scaly and caducous in *L. acida* (Fig. 2). The 5-lobed perianth has cucculate apices and in the bud condition the adjacent lobes are united by wedge-shaped epidermal cells. In the fruit of *L. cunninghamii* the perianth lobes become strongly reflexed (Fig. 102), while in *L. acida* they show a marked convergence (Fig. 105).

There are five antiphyllous stamens with short filaments and dorsifixed anthers. Next to the stamens lies a 5-lobed epigynous disc. Between the androecium and the perianth arise unicellular epidermal hairs which are richly cytoplasmic and have a slightly broad base containing the nucleus (Figs. 3, 4). Ewart (1892) writes " . . . and in some closely allied genera, e.g. *Leptomeria*, the hairs are said to actually penetrate the loculi of the anther and to emerge on the other side ", but I have not been able to confirm this statement.

The gynaecium is inferior, and the ovarian cavity is 5-chambered at the base but unilocular above (Figs. 11-13). The central placental column bears five pendulous ovules of which only one reaches maturity while the others abort. The style is short and solid terminating in a 5-fid stigma. By the time megasporogenesis is completed, the space between the ovary wall and the placental column is completely filled up by the enlargement of the placental-ovular complex. The epidermal cells of the dome-shaped placental column and those lining the upper part of the ovarian cavity enlarge, and become interlocked (Figs. 5, 6). All the organs of the flower contain sphaeraphides and tannin occurs in the hypodermal cells of the receptacle and in some cells of the perianth. Prior to fertilization, the tissue around the placental vascular supply shows abundant starch.

Organogeny — The floral primordium arises as a small protuberance in the axil of the leafy bract (Fig. 7A). Along its periphery appear the perianth lobes (usually five but sometimes only four), followed by the epiphyllous stamens (Fig. 6B) and lastly the five carpels (Fig. 6C) and the central placental column (Fig. 6D). Due to the converging growth of the carpellary primordia, the stylar canal becomes completely obliterated.

Vascular Supply to the Flower — The vascular supply of the flower is essentially similar in both the species. The



Figs. 1-7 — Figs. 1 and 3-7. *Leptomeria cunninghamii* and Fig. 2. *L. acida*. Figs. 1, 2. Flowering twigs of *L. cunninghamii* and *L. acida* respectively. × 10. Figs. 3, 5. L.s. flower buds. × 30. Fig. 4. Magnified portion, marked A in Fig. 3, showing long hair. × 309. Fig. 6. Portion marked B in Fig. 5; note the interlocked epidermal cells between the placental column and the ovary wall. × 398. Fig. 7. L.s. inflorescence of *L. cunninghamii* showing development of floral organs. × 398

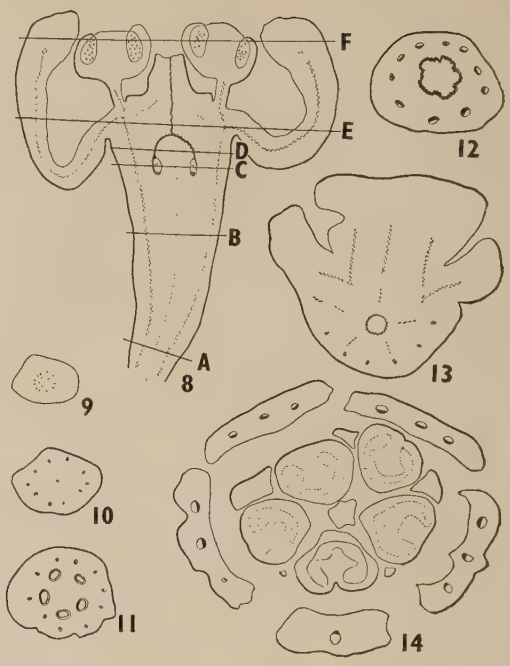
following description applies particularly to *L. cunninghamii.*

The base of the pedicel shows a ring of ten bundles from which five weak traces are given off on the inner side (Figs. 8, 9). These are connected to the central strand which supplies the placental column (Fig. 8). The original stelar ring continues as such up to the apex of the ovarian cavity (Figs. 10-12) after which several traces depart to the style (Figs. 8, 13). Of the ten stelar bundles, the five alternating ones form the combined supply to the androecium and perianth. Subsequently, they separate and enter the respective organs (Fig. 8). The remaining five bundles bifurcate and the two branches of each form the laterals of the adjacent perianth lobes (Fig. 13). Thus, each perianth lobe receives three traces of which only the median trace reaches up to the apex (Fig. 14).

Microsporogenesis — At the microspore mother cell stage the wall of the anther comprises the epidermis, endothecium, a single middle layer and the glandular tepetum (Figs. 15, 16). The enlargement of the anther is accompanied by the stretching and flattening of the epidermal cells. With the onset of meiosis the tapetal cells become binucleate, the middle layer collapses, and the cells of the endothecial layer elongate radially (Fig. 24). They become vacuolated and fibrous thickenings appear only after the formation of the microspores (Fig. 25).

A feature of special interest is the breaking down of the partition between the pollen sacs resulting in the formation of a common cavity (Fig. 25). Along the inner boundary of the connective, the apical part of the anther consists of thin-walled, small cells and due to their disintegration a horse-shoe-shaped stomium is formed (Fig. 26).

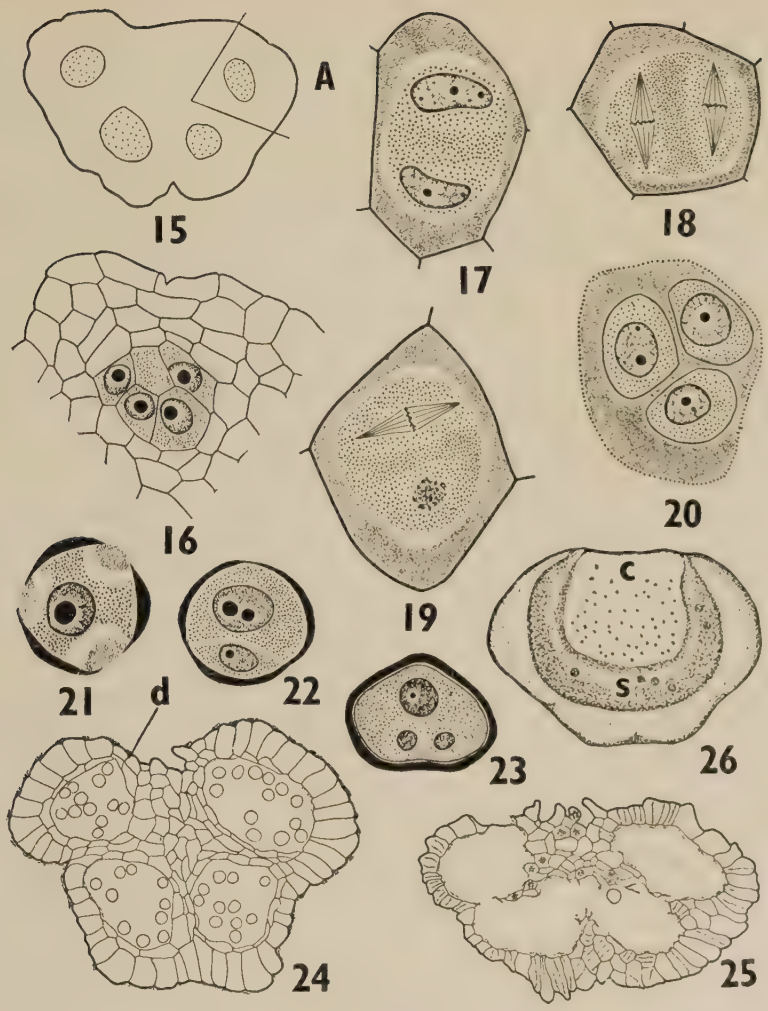
Just before Meiosis I, the cytoplasm of the microspore mother cells recedes from the walls and a special mucilaginous wall is secreted. The reduction divisions are simultaneous and during Meiosis II the spindles may be arranged parallel or at right angles to each other (Figs. 17-19). Cytokinesis takes place by furrowing and is preceded by the aggregation of the cytoplasm around the nuclei. The microspore tetrads are usually tetrahedral (Fig. 20) but sometimes decussate. The enlargement of the microspores is accompanied by the gradual absorption of the special mucilaginous wall. Then the original mother wall breaks down releasing the microspores.



Figs. 8-14 — *L. cunninghamii.* Fig. 8. L.s. flower bud. × 45. Figs. 9-14. Transections at levels marked A, B, C, D, E and F in Fig. 8. × 45.

Male Gametophyte — The wall of the microspore differentiates into a thick exine and a thin intine. The pollen grain is globose with three germ pores (Fig. 21). The microspore nucleus divides producing a smaller generative and a larger vegetative cell (Fig. 22). In *L. cunninghamii* the generative cell divides before the pollen is shed (Fig. 23), but in *L. acida* shedding occurs at the 2-celled stage. In some dehisced anthers the pollen grains had germinated *in situ.* Germinating pollen grains were also noticed on the disc.

Ovule — The ovules arise laterally from the apex of the placental column (Fig. 27). In *L. cunninghamii* they bend downwards till the tips face the base

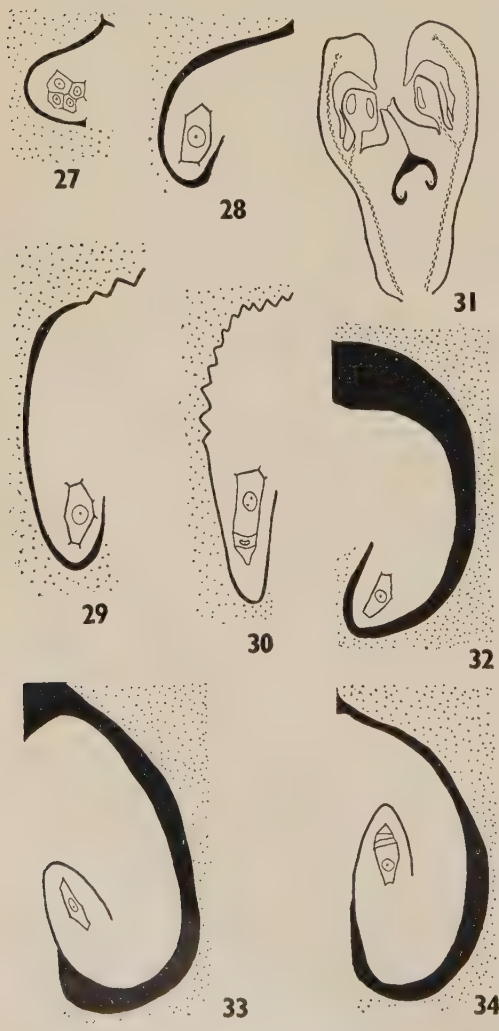


FIGS. 15-26 — Figs. 15-23 and 26. *L. cunninghamii* and Figs. 24, 25. *L. acida* (*c*, connective; *d*, region of dehiscence; *s*, stomium). Fig. 15. T.s. anther at microspore mother cell stage. × 330. Fig. 16. Enlargement of anther lobe marked A in Fig. 15. × 748. Fig. 17. Dyad. × 1496. Figs. 18, 19. Meiosis II. × 1496. Fig. 20. Tetrahedral tetrad. × 1496. Figs. 21-23. One, two and three-celled pollen grains. × 1496. Fig. 24. T.s. mature anther. × 330. Fig. 25. Same, dehisced condition. × 330. Fig. 26. Top view of dehisced anther. × 330.

of the ovarian cavity (Figs. 28-30). In *L. acida*, on the other hand, the bending continues till they become anatropous with their tips directed towards the style (Figs. 31-34). The ovules project into pouches so that in a cross-section of the basal region, the ovary appears 5-chambered (Fig. 11).

The ovules in the Santalaceae have been generally believed to be ategmic (Engler & Prantl, 1889; Modilewski, 1928; Iyengar, 1937; Schaeppi, 1942; Schaeppi & Steindl, 1937). However, in many species of *Thesium* the tip of the ovule shows a small depression (Warming, 1878; Guignard, 1885; Schulle, 1933; Rutishauser, 1937) and Goebel (1933) suggested that this depression may be regarded as the vestige of a micropyle. According to Paliwal (1956), " . . . in *Osyris, Santalum*

and *Thesium* the nucellus is very narrow and is represented by a few layers of elongated and richly cytoplasmic cells only. The remaining bulk of tissue present laterally to this narrow nucellus represents the integumentary tissue." My preparations of *Leptomeria* do not show any apical depression or any differentiation of cells. Occasionally, a single elongated cell is seen at the extreme apex (Figs. 41, 53) and probably this alone represents the much reduced nucellus. The adjacent integumentary cells undergo periclinal divisions but do not form any micropyle.



FIGS. 27-34 — Figs. 27-30. *L. cunninghamii*; Figs. 31-34. *L. acida*. Figs. 27-30 and 32-34. Stages showing progressive development and curvature of ovules. × 304. Fig. 31. Longisection of a bud. Fig. 32. Portion of Fig. 31 enlarged to show ovule at megaspore mother cell stage. × 38.

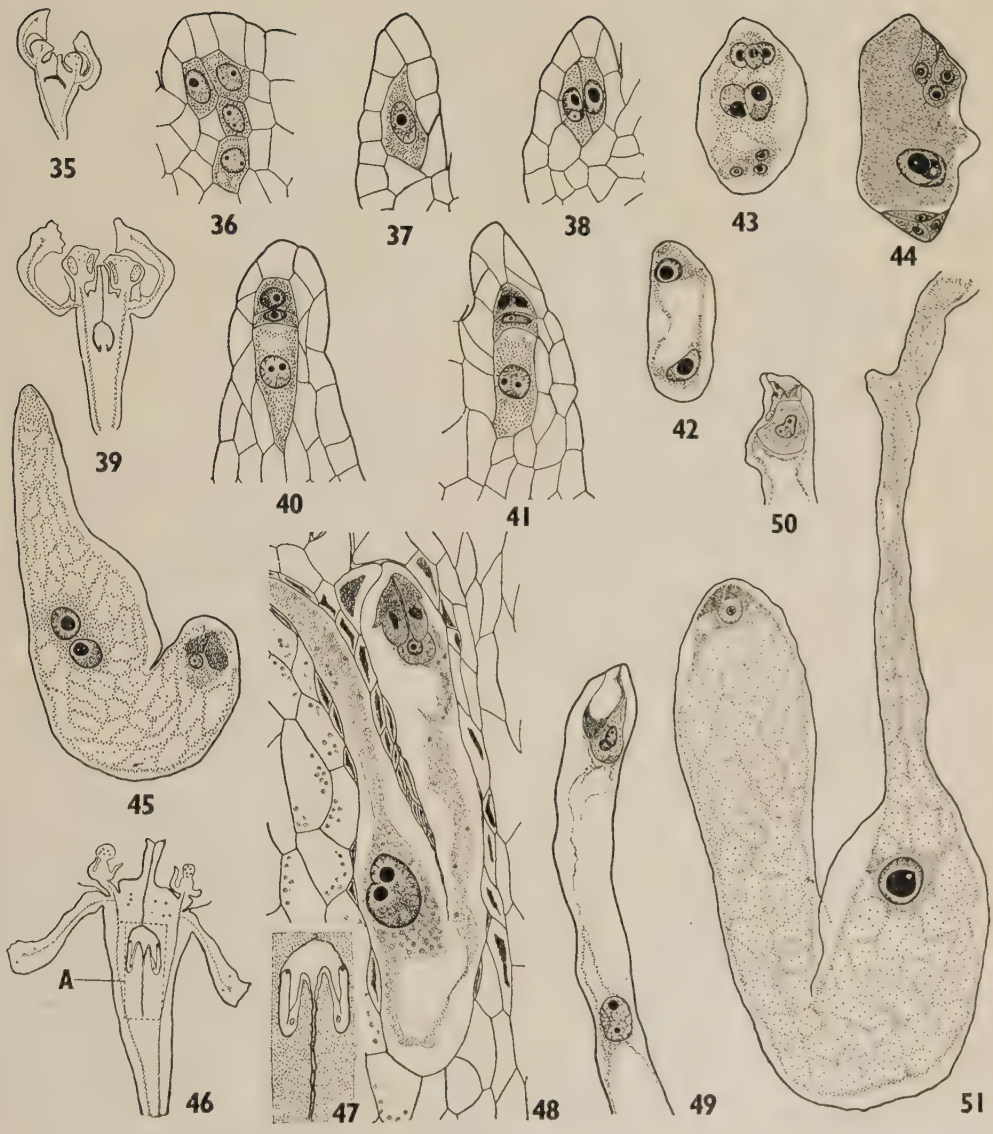
The developing embryo sacs absorb the ovulary tissue. At the 4-nucleate stage the degeneration is well marked on the sides (Fig. 59) and the apex, and finally the tip of the embryo sac projects beyond the ovule (Fig. 61). From the chalazal end of the embryo sac arises a caecum which consumes the basal ovulary tissue and makes its way into the placental column.

In *L. cunninghamii* the ovules are rather small and even at the megaspore mother cell stage they are closely pressed against the ovary wall. The ovules are much larger in *L. acida* and in this species there is an appreciable space between the ovary wall and the ovule (Figs. 32-34).

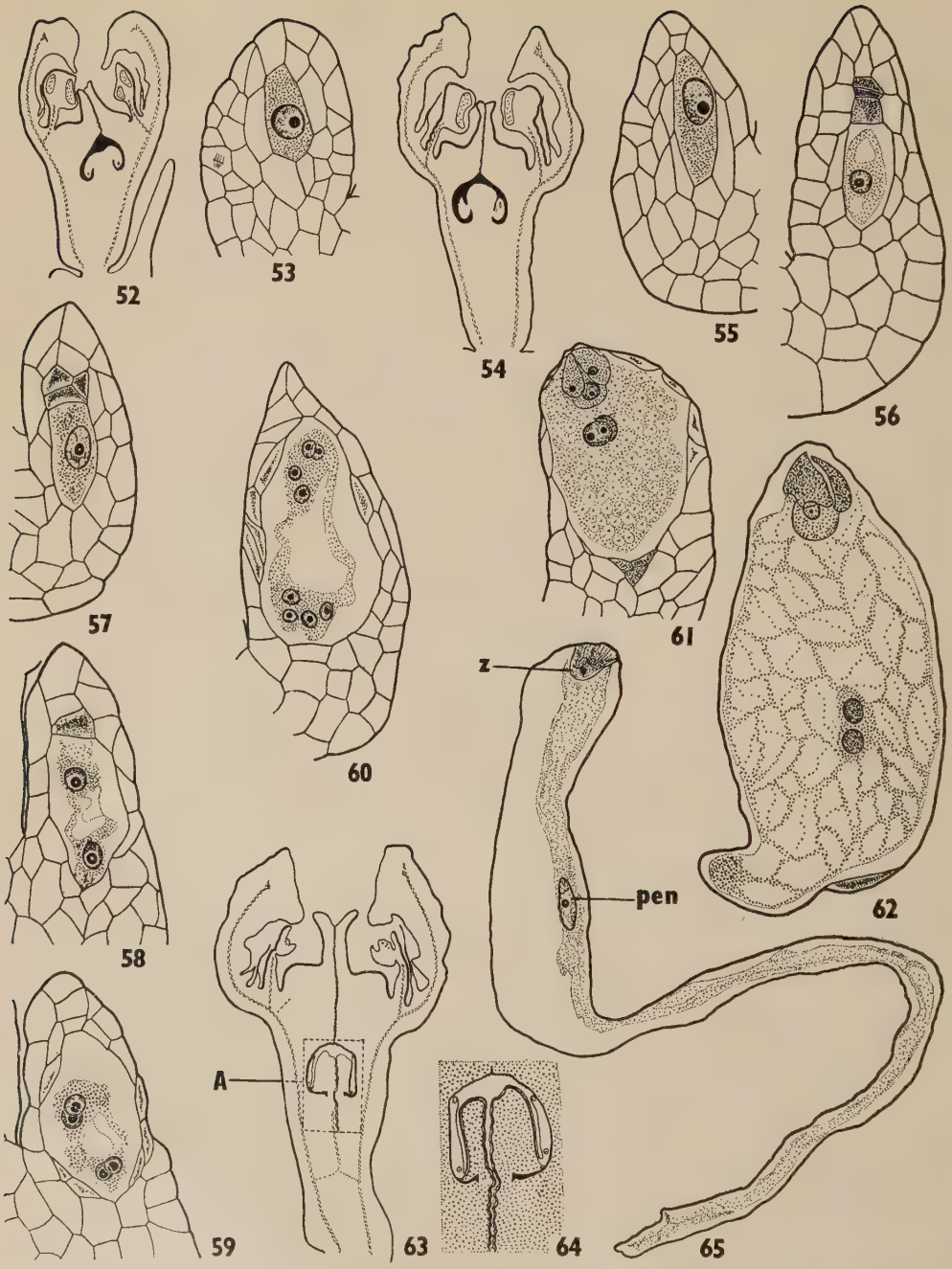
The central portion of the placental column consists of narrow and elongated cells but in the outer region they are large and polygonal.

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE — A group of hypodermal archesporial cells differentiates in the young ovules (Figs. 35, 36), but generally only one (Fig. 37) or rarely two (Fig. 38) function as megaspore mother cells. In *L. cunninghamii* the megaspore mother cell gives rise to a linear row of three cells (Figs. 39, 40) of which the upper two degenerate while the basal gives rise to the embryo sac. The upper cell of the triad represents the undivided dyad cell which is further confirmed by the fact that sometimes it shows two nuclei (Fig. 41). A similar condition has also been reported in *Thesium* (Rutishauser, 1937; Rao, 1942) and in *Santalum album* (Paliwal, 1956).

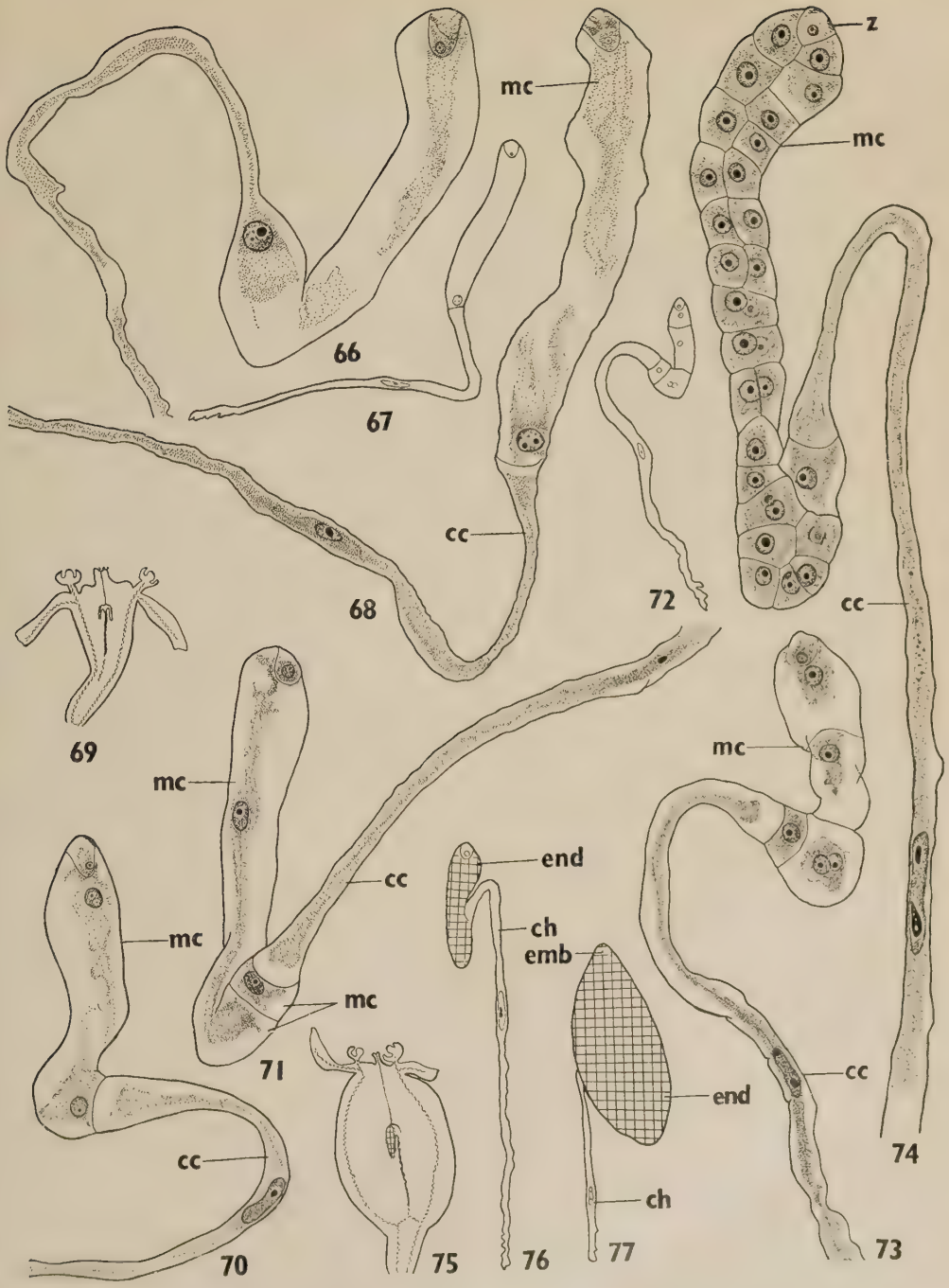
In *L. acida* both linear and T-shaped tetrads are common (Figs. 56, 57). As usual the upper three megaspores degenerate and the chalazal produces the gametophyte. It enlarges considerably followed by three nuclear divisions, resulting in two, four and eight-nucleate stages (Figs. 42, 43, 58-60). The organized gametophyte contains the egg apparatus, two polar nuclei and three antipodal cells



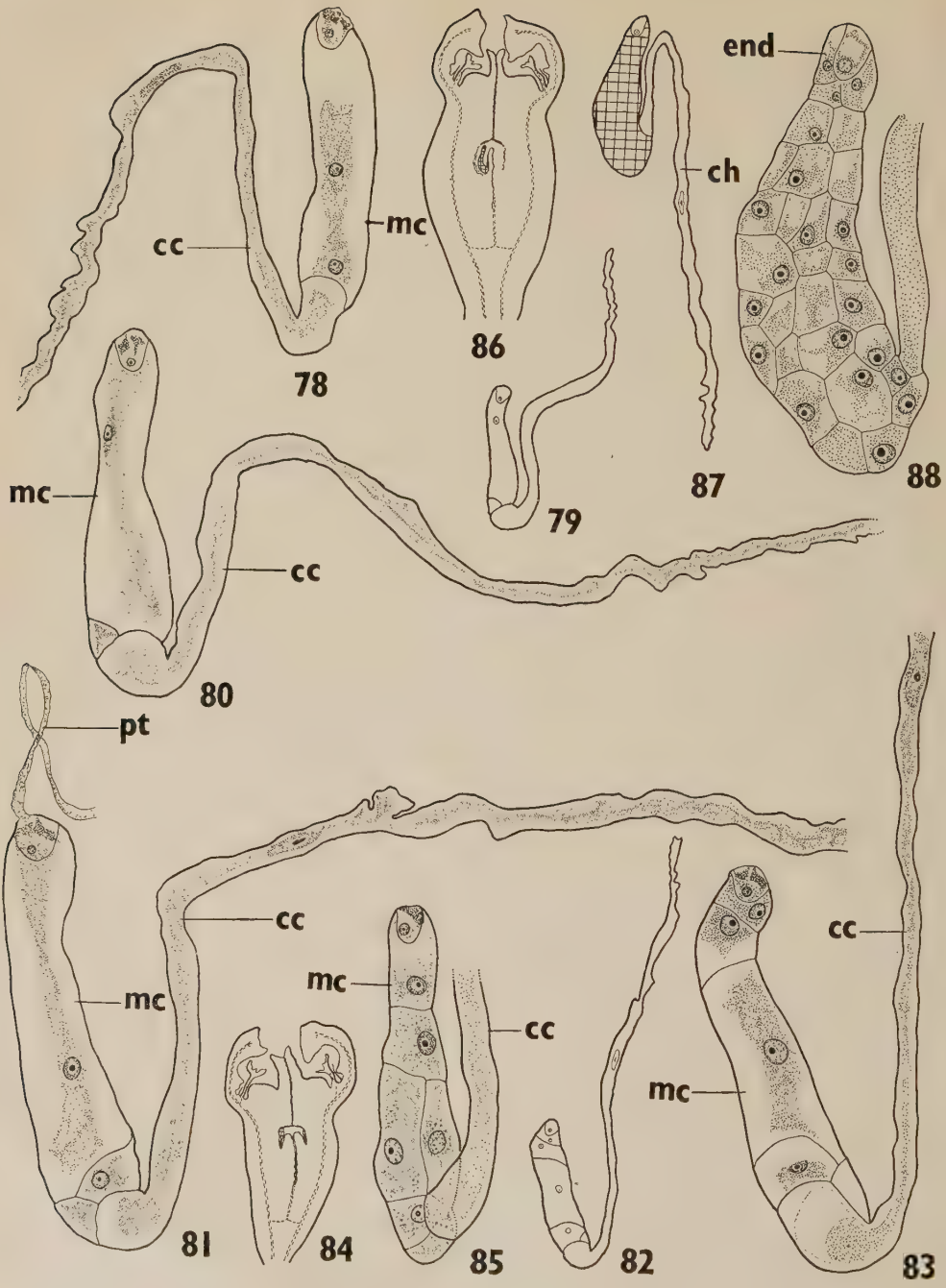
FIGS. 35-51 — *L. cunninghamii*. Fig. 35. L.s. bud. × 26. Fig. 36. Ovule enlarged from Fig. 35 showing multicelled archesporium. × 502. Figs. 37, 38. Longisection of ovules with one and two megaspore mother cells respectively. × 502. Fig. 39. Outline diagram for Figs. 40 and 41. × 26. Figs. 40, 41. Triads (explanation in text). × 502. Figs. 42, 43. Two and eight-nucleate embryo sacs. × 502. Fig. 44. Organized gametophyte. × 502. Fig. 45. Whole mount of elongating gametophyte; the antipodal cells have degenerated. × 502. Fig. 46. Longisection of flower at mature embryo sac stage. × 26. Fig. 47. Enlargement of portion marked A in Fig. 46. × 51. Fig. 48. Embryo sac showing polar fusion. × 502. Figs. 49, 50. Syngamy. × 502. Fig. 51. Embryo sac with zygote and primary endosperm nucleus. × 502.



FIGS. 52-65 — *L. acida.* (*pen*, primary endosperm nucleus; *z*, zygote). Fig. 52. L.s. flower; outline diagram for Fig. 53. × 41. Fig. 53. L.s. ovule showing sporogenous cell. × 741. Fig. 54. L.s. flower; outline diagram for Fig. 55. × 41. Fig. 55. L.s. ovule with megaspore mother cell. × 741. Figs. 56, 57. Linear and T-shaped tetrads. × 741. Figs. 58-60. Two, four and eight-nucleate embryo sacs. × 741. Fig. 61. Organized gametophyte. × 741. Fig. 62. Same, advanced stage. × 741. Fig. 63. Longisection of flower at mature embryo sac stage. × 41. Fig. 64. Enlargement of portion marked A in Fig. 63. × 76. Fig. 65. Whole mount of fertilized embryo sac. × 741.



FIGS. 66-77 — *L. cunninghamii* (*cc*, chalazal chamber; *ch*, chalazal haustorium; *end*, endosperm; *mc*, micropylar chamber; *z*, zygote). Fig. 66. Fertilized embryo sac; synergids have degenerated. × 253. Fig. 67. Outline diagram for Fig. 68. × 58. Fig. 68. Two-celled endosperm showing chalazal and micropylar chamber. × 253. Fig. 69. L.s. flower at stages represented in Figs. 68 and 70. × 26. Fig. 70. Binucleate micropylar endosperm chamber and uninucleate chalazal chamber. × 253. Fig. 71. Endosperm showing two-celled micropylar and uninucleate chalazal chamber. × 253. Fig. 72. Outline diagram for Fig. 73. × 58. Fig. 73. Four-celled micropylar (one of the cells is binucleate) and uninucleate chalazal chamber. × 253. Fig. 74. Micropylar chamber shows biseriate endosperm and zygote; nucleus of the chalazal chamber is binucleolate. × 253. Fig. 75. L.s. young fruit indicating the position of endosperm. × 26. Figs. 76, 77. Micropylar chamber contains cellular endosperm (cross-hatched); note the uninucleate chalazal haustorium. × 253.



FIGS. 78-88 *L. acida*. (*cc*, chalazal chamber; *ch*, chalazal haustorium; *end*, endosperm; *mc*, micropylar chamber; *pt*, pollen tube). Fig. 78. Two-celled endosperm showing binucleate micropylar chamber and portion of the chalazal chamber. × 760. Fig. 79. Outline diagram for Fig. 80 (change in shape is due to displacement during remounting). × 68. Fig. 80. Later stage, the micropylar chamber shows two cells – a basal lateral cell and an upper larger cell. × 294. Fig. 81. Three-celled micropylar chamber and uninucleate chalazal chamber. × 294. Fig. 82. Index figure for Fig. 83. × 68. Fig. 83. Five-celled micropylar chamber. × 294. Figs. 84, 86. Longisections of young fruits. × 30. Figs. 85, 87. Endosperms enlarged from ovules on left-hand side in Figs. 84 and 86. × 294. Fig. 88. Micropylar chamber showing cellular endosperm and zygote. × 294.

(Figs. 44, 61). There are abundant starch grains which often mask the nuclei. The synergids are small and hooked (Fig. 48) and degenerate before fertilization.

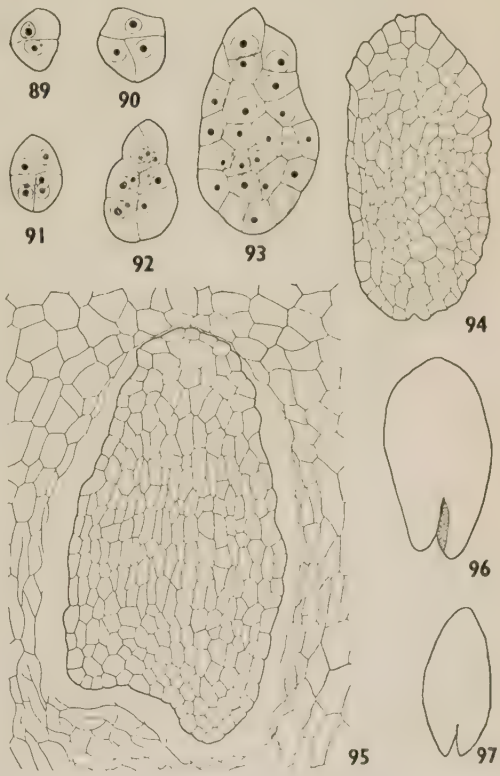
In *L. cunninghamii* the tip of the embryo sac grows beyond the ovule, bends upwards, and extends up to the base of the style (Figs. 45-47). Meanwhile, just above the level of the antipodal cells, a caecum develops at the chalazal end (Figs. 45, 62) and after traversing the ovule it bends and enters the placental column (Figs. 46, 47). Finally, the outline of the embryo sac appears like an inverted N, the chalazal arm being invariably longer than the micropylar (Fig. 47). The polar nuclei are situated in the central part of the embryo sac where they fuse to form the secondary nucleus (Figs. 48, 49, 62). By this time most of the starch grains in the embryo sac have been digested. In Fig. 49 a male gamete is seen next to the egg nucleus while triple fusion is already over. Fig. 50 shows the fusion of the male and female nuclei.

The shape of the mature embryo sac in *Leptomeria* is comparable to that of *Santalum*. An important difference, however, is that whereas in *Santalum* the tip of the embryo sac starts elongating at the 4-nucleate stage, in *Leptomeria* the elongation takes place only in the mature embryo sac.

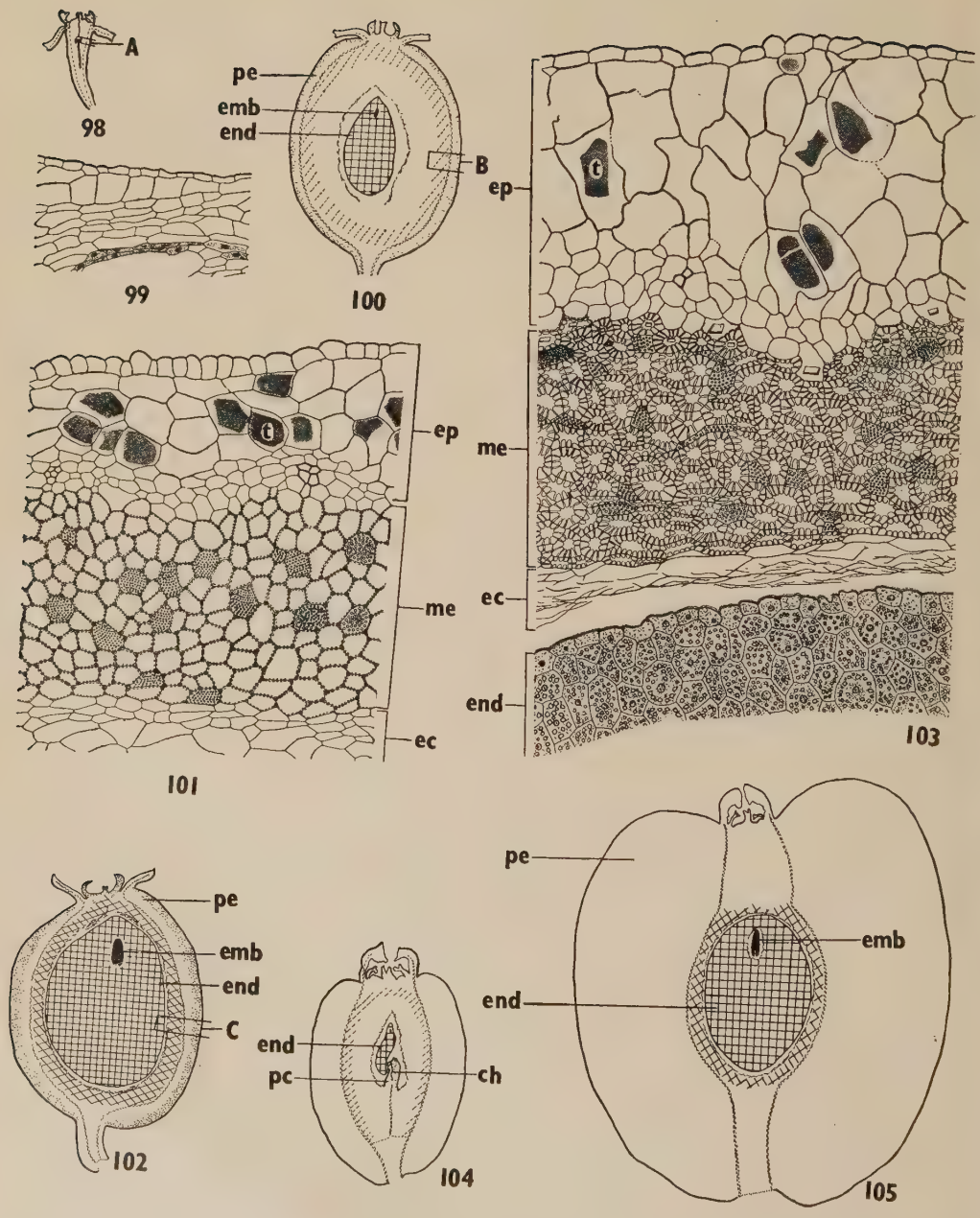
Endosperm — The primary endosperm nucleus divides much earlier than the zygote, and the first division is followed by a curved wall forming a longer chalazal (*cc*) and a shorter micropylar (*mc*) chamber (Figs. 67, 68). The former remains uninucleate and functions as a haustorium (Figs. 69-72, 78-83). The nucleus moves to the centre, becomes elongated and the nucleolus may undergo fragmentation (Figs. 74, 77). The haustorium (*ch*) shows several folds on its surface and reaches almost to the base of the ovary (Figs. 75, 84, 86). It comes in close contact with the vascular strand of the placental column and probably helps in the translocation of the food.

The nuclear division in the micropylar chamber (*mc*) is not immediately followed by a wall (Figs. 70, 78). One of the daughter nuclei moves upwards while the other remains close to the partition wall between the micropylar and the chalazal chambers. Eventually it becomes delimited by a wall which may be oriented either transversely (*L. cunninghamii*, Fig. 71) or obliquely (*L. acida*, Fig. 80). It undergoes no further divisions and the entire endosperm is produced by repeated divisions of the upper larger cell of the micropylar chamber (Figs. 73-77, 81-83, 85, 87, 88).

In both species endosperm formation may commence in all the five ovules of an ovary but ultimately it reaches maturity in only one of them. In the remaining



Figs. 89-97 - Figs. 89-96. *L. cunninghamii* and Fig. 97. *L. acida*. Figs. 89, 90. Two and three-celled proembryos. × 430. Figs. 91, 92. Quadrant and octant stages; only one nucleus was seen in one of the terminal cells in Fig. 92. × 430. Figs. 93, 94. Later stages in development of embryo. Fig. 93. × 430 and Fig. 94. × 245. Fig. 95. Heart-shaped embryo. × 245. Figs. 96, 97. Whole mounts of dicotyledonous embryos. × 44.



FIGS. 98-105 — Figs. 98-103. *L. cunninghamii* and Figs. 104, 105. *L. acida*. (*ch*, chalazal haustorium; *ec*, endocarp; *emb*, embryo; *end*, endosperm; *ep*, epicarp; *me*, mesocarp; *pc*, placental column; *pe*, pericarp). Fig. 98. L.s. flower at mature embryo sac stage. × 9. Fig. 99. Enlargement of portion marked A in Fig. 98. × 125. Fig. 100. L.s. young fruit. × 9. Fig. 101. Magnified view of portion marked B in Fig. 100. × 125. Fig. 102. L.s. mature fruit. × 9. Fig. 103. Enlargement of portion marked C in Fig. 102. × 125. Figs. 104, 105. L.s. young and mature fruits of *L. acida*. × 9.

ovules the endosperm becomes arrested at the four or six-celled stage.

EMBRYOGENY — The zygote undergoes a long period of rest and shows considerable quantities of starch. The first division is transverse giving rise to a basal and a terminal cell (Fig. 89). The basal cell produces a three to four-celled suspensor. The terminal cell divides twice, leading to the formation of a quadrant (Figs. 90, 91). The third division is in the horizontal plane and results in an octant (Fig. 92). Further transverse and longitudinal divisions give rise to the globular stage (Figs. 93, 94), after which the two cotyledons, radicle and plumule are differentiated in the usual way (Figs. 95-97).

PERICARP — At the mature embryo sac stage the ovary wall is composed of 8-10 layers of cells (Figs. 98, 99). The outer epidermis is followed by a layer of radially elongated cells with some granular contents, five to six layers of narrow cells and lastly the inner epidermis (Fig. 99). Due to the divisions of the cells of the middle region the pericarp becomes 35 to 40-layered. At the globular stage of the proembryo it is distinguishable into three zones: an epicarp (*ep*) of enlarged paren-

TABLE 1

	SANTALUM	THESIUM	OSYRIS	LEPTOMERIA
Flower	Hermaphrodite, tetra- or pentamerous	Hermaphrodite, tetra- or pentamerous	Unisexual, trimerous	Hermaphrodite, pentamerous
Pollen Grains	2 or 3-celled	2-celled	2-celled	2 or 3-celled
Ovary	Semi-inferior	Inferior	Inferior	Inferior
Placentum	Short and beaked	Long and twisted	Short and without beak	Short and without beak
Ovule	3, ortho- or anatropous, micropyle indistinct	3, ortho- or anatropous, micropyle distinct	3, anatropous, micropyle indistinct	5, ortho- or anatropous, micropyle indistinct
Embryo Sac	Tip of embryo sac extends beyond the ovule and reaches up to the base of the style	Tip of embryo sac does not extend beyond the ovule.	Tip of embryo sac does not extend beyond the ovule	Tip of embryo sac extends beyond the ovule and reaches up to the base of the style
	Chalazal caecum present	Chalazal caecum present	Chalazal caecum present	Chalazal caecum present
Endosperm	Helobial; endosperm formation starts in all the three ovules but reaches maturity only in one of them	Cellular; endosperm formation starts only in one of the three ovules	Cellular; endosperm formation starts only in one of the three ovules	Cellular; endosperm formation starts in all the five ovules but reaches maturity only in one of them
	Cell formation in the micropylar chamber starts from the base	—	—	Cell formation in the micropylar chamber starts from the base
Embryo	First division transverse	First division transverse	First division transverse	First division transverse
Suspensor	Present	Absent	Absent	Present

chymatous cells containing tannin, a mesocarp (*mc*) of smaller cells which develops sclerenchymatous thickenings, and a broad endocarp (*ec*) of thin-walled cells (Figs. 100, 101). The latter is consumed by the aggressive endosperm so that at maturity the pericarp consists of the epicarp, mesocarp and the degenerated remains of the endocarp (Figs. 102, 103). In *L. acida* the cells of the epicarp become much elongated.

Fruit and Seed — The fruit is an ellipsoidal nut. It is hard and ribbed in *L. cunninghamii*, but glossy and soft in *L. acida* (Figs. 104, 105). As compared to the size of the endosperm the mature embryo is much smaller (Figs. 102, 105). The endosperm cells are isodiametric and contain reserves of fatty substances. A thin layer of cuticle covers the epidermal cells of the endosperm (Fig. 103). During maturation the embryo consumes the adjoining endosperm cells.

Systematic Position — On the basis of structure of the gynaecium, Engler & Prantl (1889) classified the family Santalaceae into three tribes — Anthoboleae, Osyrideae and Thesieae—and included *Leptomeria* in the Osyridae along with *Osyris*, *Santalum* and *Comandra*. Most taxonomists agree with this assignment of *Leptomeria* (Pilger, 1935; Wettstein, 1935; Engler & Diels, 1936). Taking the placental-ovular-complex into consideration, Van Tieghem (1896) erected four tribes — Santalées, Osyridées, Thesiées and Comandrées — and *Leptomeria* was placed in the Santalées along with *Santalum*. Smith & Smith (1942), who have investigated the floral anatomy of the Santalaceae, are of the opinion that " . . . *Leptomeria*, *Choretrum*, . . . are more closely related to members of Thesieae than to those of Osyrideae."

The floral morphology and embryology of *Leptomeria*, *Osyris* (Osyrideae), *Santalum* (Santaleae) and *Thesium* (Thesieae) are compared in Table 1 (*see* page 31).

As can be judged from this table, Smith & Smith's (1942) suggestion that *Leptomeria* is more closely related to Thesieae does not find support on embryological grounds.

There are obvious similarities between *Leptomeria* and *Santalum* in flower structure, pollen grains, ovule, shape and mode of elongation of the embryo sac and development of the endosperm. Therefore, Van Tieghem's (1896) assignment of *Leptomeria* to the tribe Santalées appears to be justified.

Summary and Conclusions

The inflorescence is racemose and the flowers are typically pentamerous. The stamens are opposite and isomeric with the perianth lobes.

The ovary is inferior and five-chambered at the base but unilocular above, and the five ovules are borne at the apical end of the central placentum.

The anther wall comprises the epidermis, fibrous endothecium, a single middle layer and the glandular tapetum. At the time of dehiscence all the microsporangia fuse and the pollen is discharged through a semi-circular stomium formed at the summit of the anther.

The reduction divisions are simultaneous, producing tetrahedral or decussate tetrads. Cytokinesis occurs by furrowing. The mature pollen grains are rounded, and have a non-sculptured exine and three germ pores. They are shed at the 2-celled stage in *L. acida* and at the 3-celled stage in *L. cunninghamii*.

The ovules do not show any clear distinction between the nucellus and the integument. A group of archesporial cells differentiates hypodermally but normally only one develops further. The archesporial cell functions directly as the megaspore mother cell. In *L. cunninghamii* only triads have been observed but in *L. acida* tetrads are also common. The development of the embryo sac conforms to the Polygonum type and the antipodal cells degenerate early.

After the organization of the nuclei, the micropylar end of the embryo sac grows out of the ovule and extends in the direction of the stylar canal. A chalazal caecum is formed slightly above the level of the antipodal cells. It enters the placental column and finally the embryo sac becomes ↳-shaped.

The first division of the primary endosperm nucleus is followed by a wall forming a chalazal and a micropylar chamber,

The former acts as a uninucleate haustorium. The micropylar chamber alone contributes to the formation of the endosperm proper. Endosperm formation may be initiated in all the five ovules of an ovary but it reaches maturity in only one of them.

The first division of the zygote is transverse. The basal cell gives rise to a small suspensor while the terminal cell produces a dicotyledonous embryo.

The endosperm is enclosed directly by the pericarp which consists of parenchymatous epicarp and stony mesocarp. The endocarp is consumed by the endosperm.

On embryological grounds there seems to be a close relationship between *Leptomeria* and *Santalum*.

It gives me great pleasure to express my gratitude to Dr B. M. Johri and Professor P. Maheshwari for their valuable suggestions and critical comments. I am also indebted to Professor H. S. McKee and Dr A. M. Baird for collecting the material.

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DEVELOPMENT OF ENDOSPERM HAUSTORIA IN SOME LEGUMINOSAE

B. M. JOHRI & SUDHA GARG

Department of Botany, University of Delhi, Delhi 8, India

The outstanding features in the ovules of the family Leguminosae are a conservative seed coat (Corner, 1951), massive suspensor (Anantaswamy Rau, 1950a, c; 1951a, f), and chalazal endosperm haustorium (Anantaswamy Rau, 1950b; 1951b, c, d, e, g; 1953; 1954; Dnyansagar, 1949; 1954a, b, c; 1957; 1958; Pantulu, 1951).

The haustorium mostly remains free nuclear although the cellular condition is known in a few cases. Its shape, size and activity differ widely. This study was undertaken to extend our knowledge on the origin, structure and behaviour of the haustorium in all the three subfamilies of the Leguminosae. A brief report has already appeared elsewhere (*see* Johri & Garg, 1956).

Materials and Methods

The following genera and species belonging to nine tribes have been studied:

LOTOIDEAE[1] (syn. PAPILIONATAE): Genisteae — *Argyrolobium flaccidum* Jaub. & Spach., *Crotalaria medicagenia* Lamk.; Galegeae — *Tephrosia villosa* Pers.; Hedysereae — *Alhagi camelorum* Fisch., *Desmodium gangeticum* DC., *D. floribundum* G. Don., *D. laburnaefolium* DC., *Zornia diphylla* Pers.; Phaseoleae — *Vigna vexillata* Benth.; Dalbergieae — *Dalbergia sissoo* Roxb.

CAESALPINIOIDEAE: Cassieae — *Cassia absus* L., *C. sophera* L.; Eucaesalpinieae — *Delonix regia* (Boj.) Raf.

MIMOSOIDEAE: Mimoseae — *Mimosa hamata* Willd., *M. pudica* L. *Prosopis juliflora* DC., *P. spicigera* L.; Acacieae — *Acacia modesta* Wall., *A. senegal* Willd., *Albizzia lebbek* Benth.

Most of the material was obtained from the Delhi Ridge and the University Botanical Garden. We are grateful to Dr H. Y. Mohan Ram for collecting *Argyrolobium* and *Desmodium floribundum* from Mussoorie, and to Messrs P. P. Khanna, Virendar Kumar and P. P. Sehgal for collecting *Vigna vexillata* and *Cassia absus* from Simla, *Desmodium laburnaefolium* from the Forest Research Institute, Dehra Dun, and *Alhagi camelorum* from Shahdara (Delhi).

Formalin-acetic-alcohol was used for fixation and the usual methods of dehydration and imbedding were followed. Sections were cut at a thickness of 12-16 μ and stained with iron-haematoxylin and with safranin-fast green. Since microtome sections do not bring out the structure of the haustorium in its entirety, whole mounts of dissections were also examined. With the latter technique it was possible to follow a close series of developmental stages of the endosperm. The whole mounts were stained with acetocarmine, mounted in a mixture of acetocarmine and glycerine (50 : 50), and sealed with canada balsam. In a few cases Delafield's haematoxylin was used for staining and Zirkle's medium for mounting.

The food reserves of the endosperm were examined with a polarizer and also tested microchemically. Starch was tested with iodine, oil with Sudan III and proteins with Millon's reagent.

Observations

GENERAL DEVELOPMENT OF THE ENDOSPERM — In most of the plants studied the ovule is anatropous, bitegminal and

1. For explanation of the validity of the name Lotoideae *see* Rehder (1945).

crassinucellar. Occasionally, however, it may be hemi- or campylotropous. The outer integument is usually devoid of a vascular supply except in a few plants, e.g. *Tephrosia villosa, Desmodium floribundum* (Fig. 23), *Delonix regia, Prosopis* (Fig. 35), *Mimosa* (Fig. 42) and *Acacia* (Fig. 47). The endosperm is Nuclear and the nuclei become peripherally distributed. Wall formation is centripetal and is usually initiated at the micropylar end just before the proembryo reaches the globular stage.

The cellular condition is generally limited only to the upper half of the embryo sac which has been designated as the endosperm proper. The cells are uninucleate but sometimes a multinucleate condition is seen in the strip of cells adjacent to the haustorium, e.g. in *Tephrosia villosa* and *Desmodium laburnaefolium* (Fig. 15).

The length of the haustorium is variable and does not bear any relation to the length of the ovule as would be clear from Table 1 which gives the respective lengths of the haustorium and the ovule at the globular stage of the proembryo.

TABLE 1 — THE PLANTS HAVE BEEN ARRANGED ACCORDING TO THE LENGTH OF HAUSTORIUM

Plant	Length of haustorium in microns	Length of ovule in microns
Delonix regia	1840	4800
Dalbergia sissoo	1440	1840
Alhagi camelorum	800	1040
Tephrosia villosa	640	880
Desmodium floribundum	480	1072
Prosopis spicigera	480	1600
Mimosa pudica	400	800
Acacia senegal	400	1520

The haustorium may be as broad as the endosperm proper or it may be very narrow and tubular. The dimensions of the haustorium and the endosperm proper at the globular stage of the proembryo are compared in Table 2.

The haustorium remains aggressive up to the late heart-shaped or even early dicotyledonous stage of the embryo. Subsequently its activity declines; the

TABLE 2

Plant	Endosperm proper		Haustorium (free nuclear)	
	L	B	L	B
Dalbergia sissoo	960	560	1840	800
Crotalaria medicagenia	288	224	1200	240
Cassia sophera	1140	320	1120	176
Cassia absus	1146	176	1125	320
Argyrolobium flaccidum	320	224	800	240
Albizzia lebbek	960	480	720	480
Prosopis juliflora	320	240	720	240
Acacia modesta	400	240	648	304
Tephrosia villosa	329	240	640	240
Prosopis spicigera	560	432	640	238
Mimosa hamata	320	224	638	192
Vigna vexillata	80	80	632	120
Desmodium floribundum	560	400	630	120
Acacia senegal	640	480	630	240
Alhagi camelorum	120	80	560	80
Desmodium laburnaefolium	1440	368	480	160
Mimosa pudica	240	240	380	240
Zornia diphylla	336	192	360	176

B = Breadth; *L* = Length; all measurements in microns.

nuclei are the first to degenerate and their outline becomes irregular or very much elongated. The cytoplasm and the degenerating nuclei aggregate either at the tip of the haustorium or in its middle. However, degeneration may set in at different stages in different plants. Gradually, as the endosperm proper fills the entire seed cavity, the haustorium becomes progressively compressed until it is no longer distinguishable.

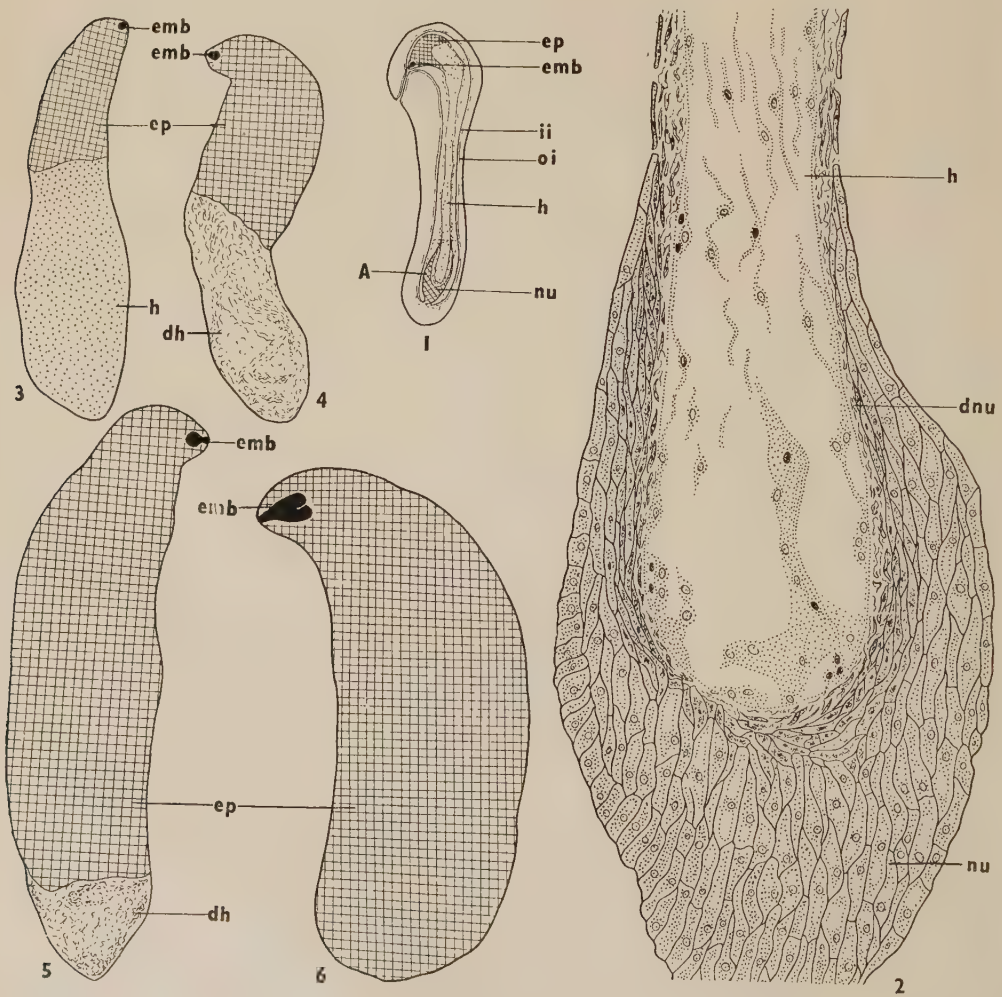
During its growth the embryo consumes the adjoining endosperm tissue of thin-walled and vacuolated cells. This process continues until most of the endosperm proper is digested and only one or two layers persist in the seed. The reserve food is mostly proteinaceous but oil is present in *Alhagi camelorum, Cassia sophera, Dalbergia sissoo* and *Vigna vexillata*. Starch occurs in small quantities in *Delonix regia*.

Structure and Development of the Haustorium — In *Dalbergia* the haustorium is a prominent sac-like structure which gradually merges into the endosperm

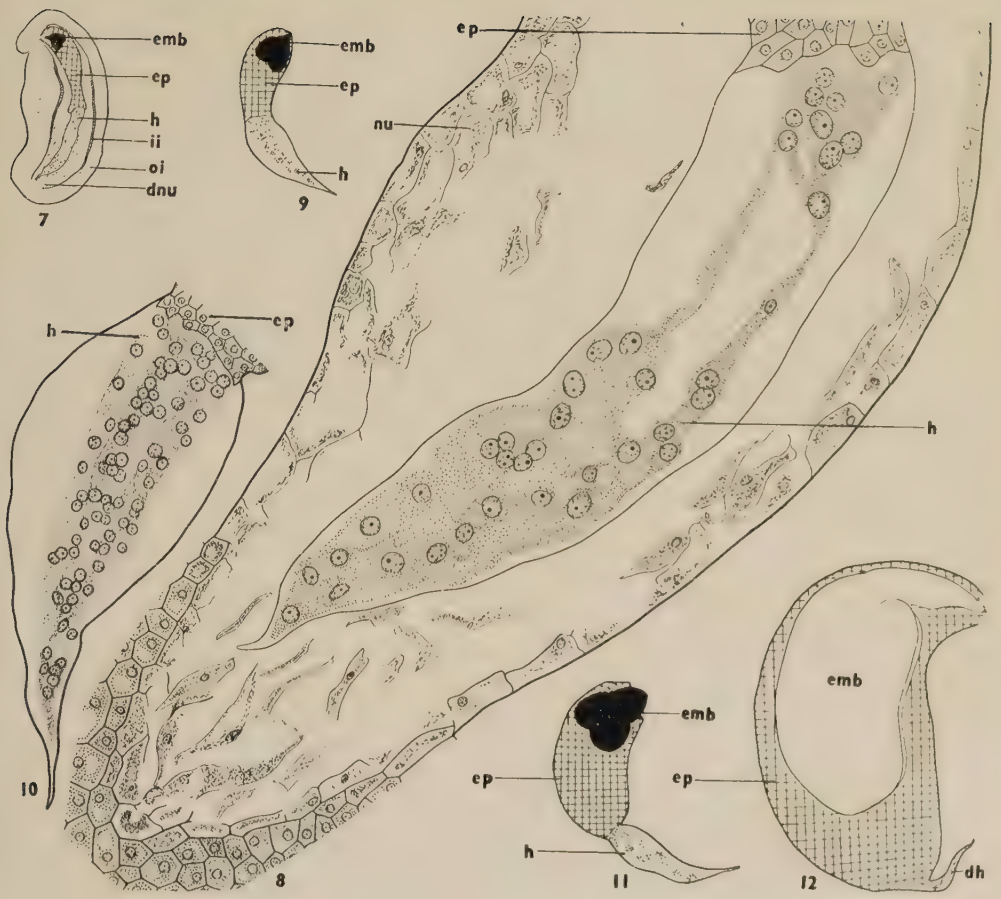
proper (Figs. 1-3). It degenerates after the early globular stage of the proembryo (Figs. 4, 5) and during the short period of its activity it consumes most of the nucellus. At the chalazal end a group of richly cytoplasmic nucellar cells form a distinct cup-shaped structure which encloses the tip of the haustorium (Figs. 1, 2). This tissue is also gradually absorbed and further extension of the haustorium is checked only by the development of a thick-walled barrier tissue at the extreme chalazal end of the ovule.

The remnants of the degenerated haustorium persist through the heart-shaped or even the early dicotyledonous stage of the embryo, but eventually become indistinguishable (Fig. 6).

The haustorium shows a similar structure in *Argyrolobium flaccidum, Vigna*



FIGS. 1-6 — *Dalbergia sissoo.* Figs. 1, 2 from microtome sections, rest from whole mounts (*dh*, degenerated remnants of haustorium; *dnu*, degenerated remnants of nucellus; *emb*, embryo; *ep*, endosperm proper; *h*, haustorium; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument). Fig. 1. L.s. young seed showing extension of the free nuclear haustorium (diagrammatic). × 29. Fig. 2. Enlarged view of portion marked *A* in Fig. 1. × 433. Figs. 3-6. Endosperm at different stages of the embryo (diagrammatic). × 29.

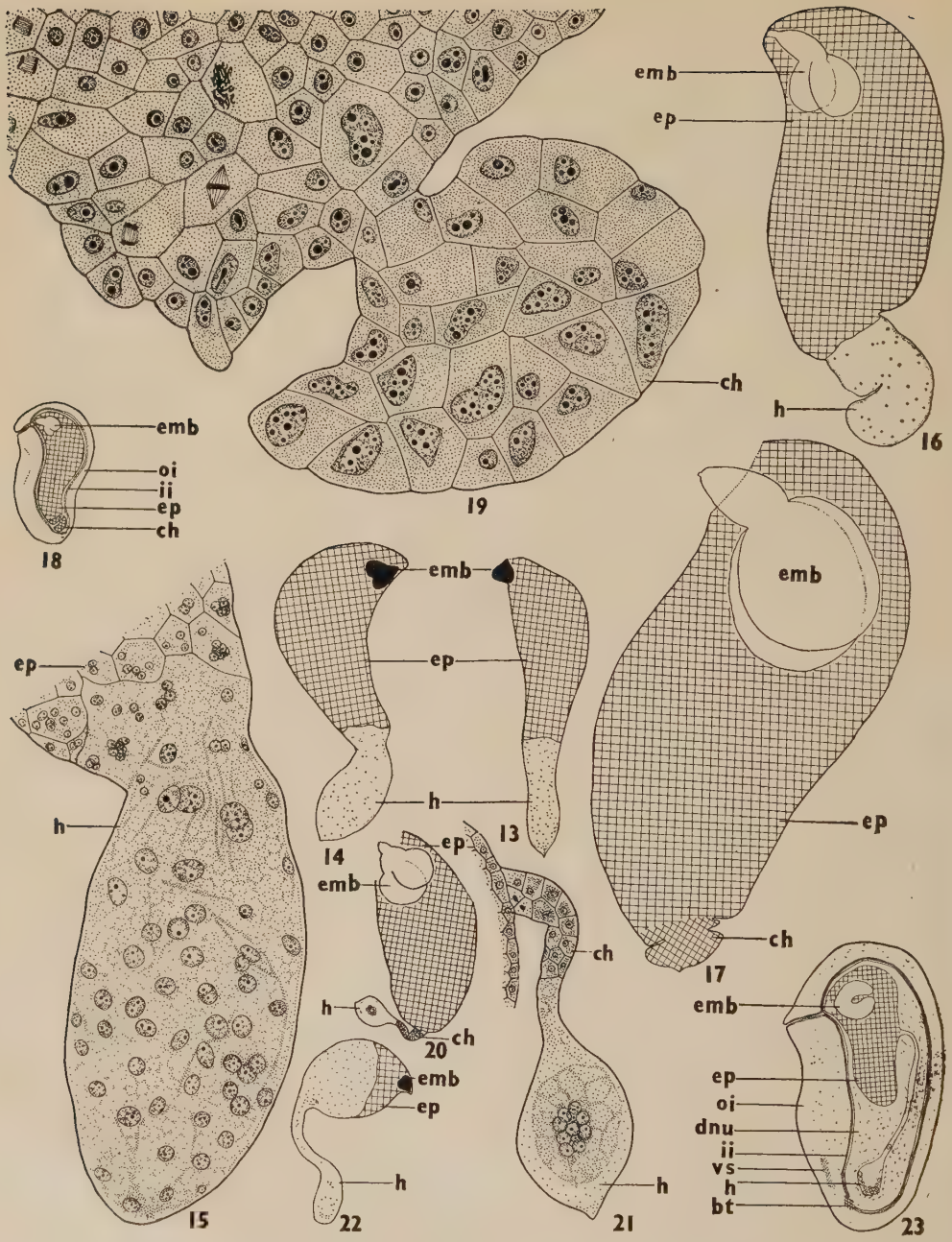


FIGS. 7-12 — *Zornia diphylla*. Figs. 7, 8 from microtome sections, rest from whole mounts; all figures diagrammatic except Figs. 8 and 10 (*dh*, degenerated remnants of haustorium; *dnu*, degenerated remnants of nucellus; *emb*, embryo; *ep*, endosperm proper; *h*, haustorium; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument). Fig. 7. L.s. young seed; the micropylar part is covered over by the outer integument only. × 38. Fig. 8. Haustorium enlarged from Fig. 7. × 366. Figs. 9, 11, 12. Endosperm at heart-shaped and dicotyledonous stages of the embryo. × 38. Fig. 10. Enlarged view of haustorium from Fig. 9. × 160.

vexillata and *Alhagi camelorum* (Lotoideae); *Delonix regia* (Caesalpinioideae); and *Acacia modesta* (Figs. 48-50) and *Albizzia lebbek* (Mimosoideae) (Figs. 51, 52) but it remains active until the early dicotyledonous stage of the embryo or even later.

The tip of the haustorium in *Tephrosia villosa* ends in a cup-shaped structure which surrounds and absorbs the nucellar tissue at the chalazal end. This is also true of *T. purpurea* (Anantaswamy Rau, 1951g). In *T. purpurea* Anantaswamy Rau reported a small tubular process arising from the lower end of the haustorium, but we did not observe any such process in *T. villosa*. During the maturation of the seed both the nucellus and the inner integument are consumed.

In *Zornia diphylla* the haustorium tapers sharply into a pointed beak-like prolongation (Figs. 9-12) and is very aggressive as borne out by the complete disorganization of the nucellus (Figs. 7, 8). Its degenerated remnants persist even after the embryo has well advanced (Fig. 12).



FIGS. 13-23 — *Desmodium*. Figs. 18, 19 and 23 from microtome sections, rest from whole mounts; all figures diagrammatic except Figs. 15, 19 and 21 (*bt*, barrier tissue; *ch*, cellular haustorium; *dnu*, degenerated remnants of nucellus; *emb*, embryo; *ep*, endosperm proper; *h*, haustorium; *ii*, inner integument; *oi*, outer integument; *vs*, vascular supply). Figs. 13-19. *D. laburnaefolium*. Figs. 13, 14, 16, 17. Endosperm at different stages of the embryo; note cellular condition of the haustorium in Fig. 17. × 38. Fig. 15. Magnified view of haustorium from Fig. 14. × 160. Fig. 18. L.s. seed. × 12. Fig. 19. Cellular haustorium from Fig. 18. × 366. Figs. 20, 21. *D. gangeticum*. Fig. 20. Endosperm at early dicotyledonous stage of the embryo. × 38. Fig. 21. Enlarged view of haustorium from Fig. 20 showing cellular condition in the upper and free nuclear in the lower portion. × 160. Figs. 22, 23. *D. floribundum*. Fig. 22. Endosperm at early heart-shaped stage of the embryo. × 38. Fig. 23. L.s. young seed showing disposition of the haustorium. × 38.

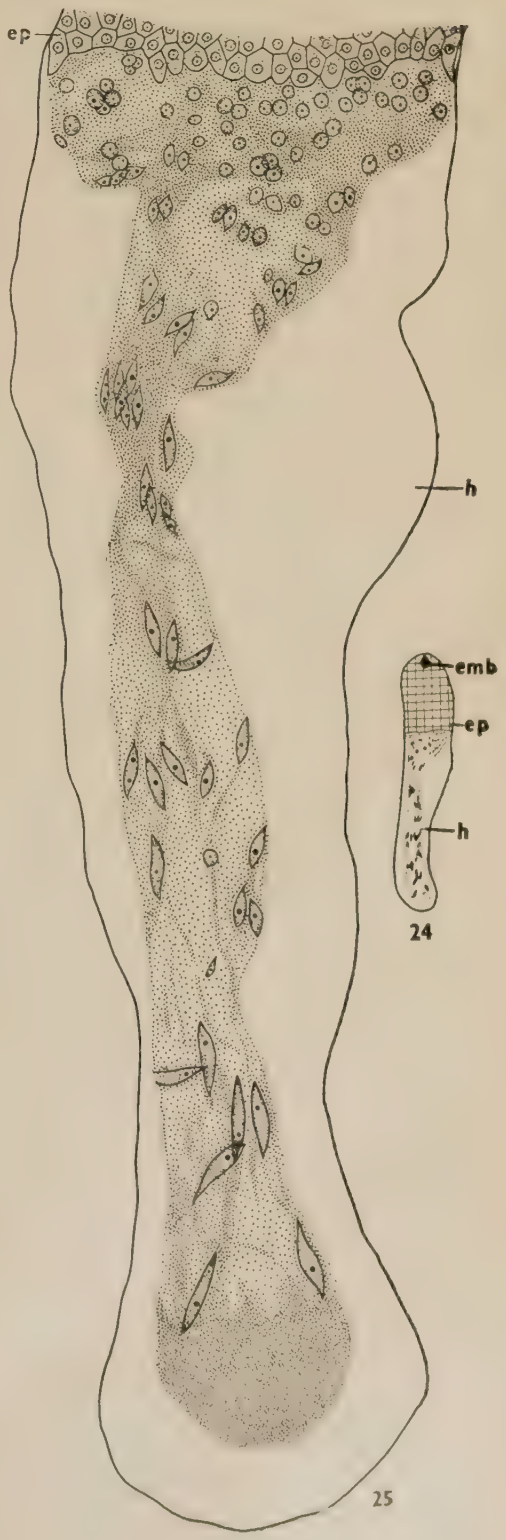
The haustorium of *Acacia senegal* (Figs. 43-47) is similar to that of *Zornia*.

In *Prosopis juliflora* the tip of the haustorium shows prominent, richly cytoplasmic, finger-like processes (Figs. 26, 27). Although these disorganize early, the haustorium remains active until the late heart-shaped stage of the embryo (Figs. 28, 29). In *P. spicigera* the haustorium is slightly constricted in the middle and has a conical tip (Figs. 31, 32). In both the species the remnants of the degenerated haustorium persist till a late stage (Figs. 30, 33, 34).

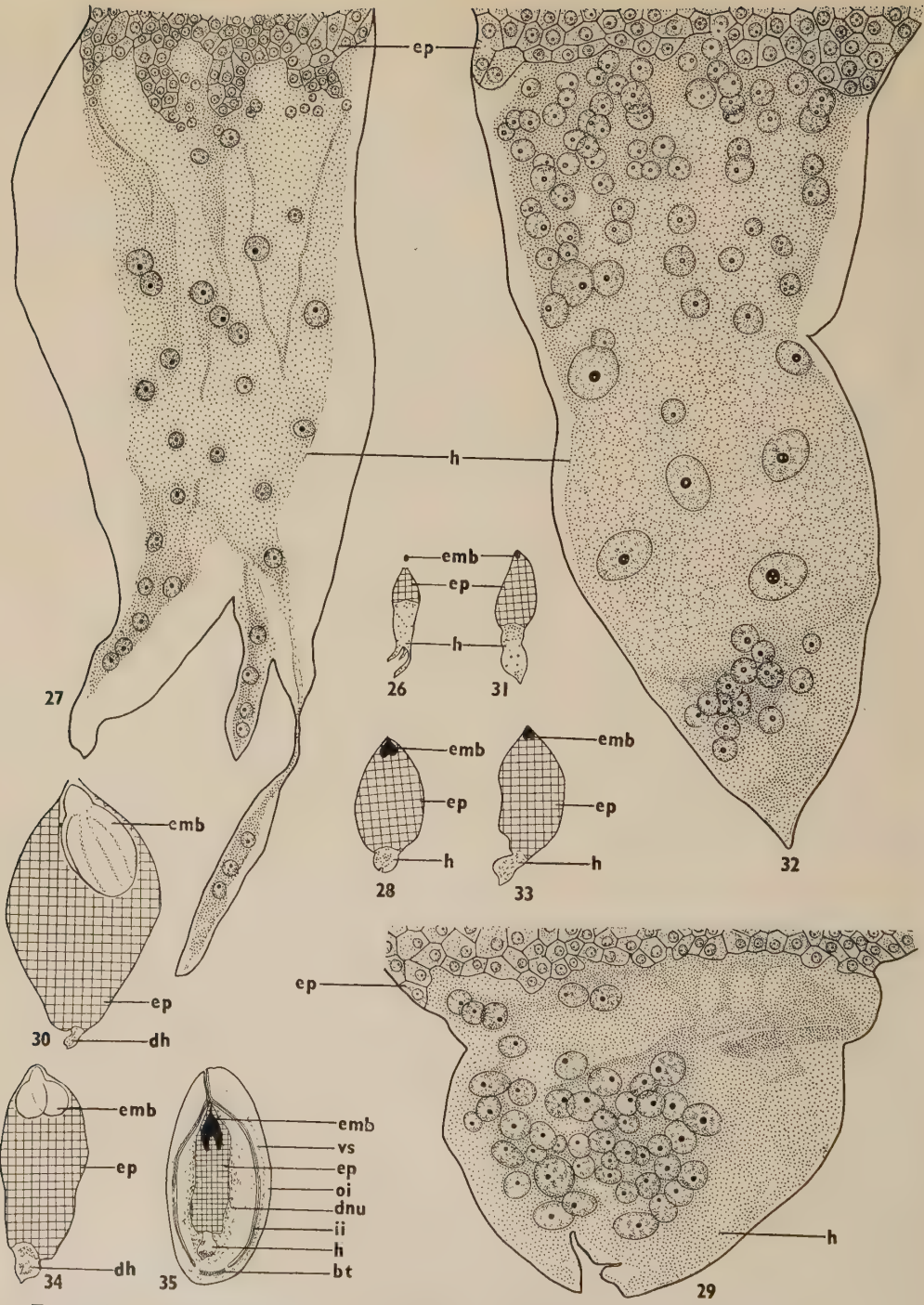
In *Mimosa hamata* and *M. pudica* also the haustorium shows a slight constriction in the middle at the early heart-shaped stage of the embryo (Figs. 36-39). Later the lower end becomes vesicular (Fig. 40), and in *M. pudica* several small processes are given off at the tip (Fig. 39). A few layers of the endosperm proper and the degenerated remnants of the haustorium persist in the nearly mature seed (Fig. 41). In *Cassia absus* (Figs. 79-81) the haustorium follows the same pattern of development.

In *Crotalaria medicagenia* the haustorium is quite different from that of the other species of this genus studied by Anantaswamy Rau (1951e, 1953). It is a fairly elongated structure which is almost uniformly broad (Figs. 75, 76). The tip is irregular and shows ridges and furrows which increase the absorptive surface. The degenerated remnants of the haustorium are represented in Fig. 78.

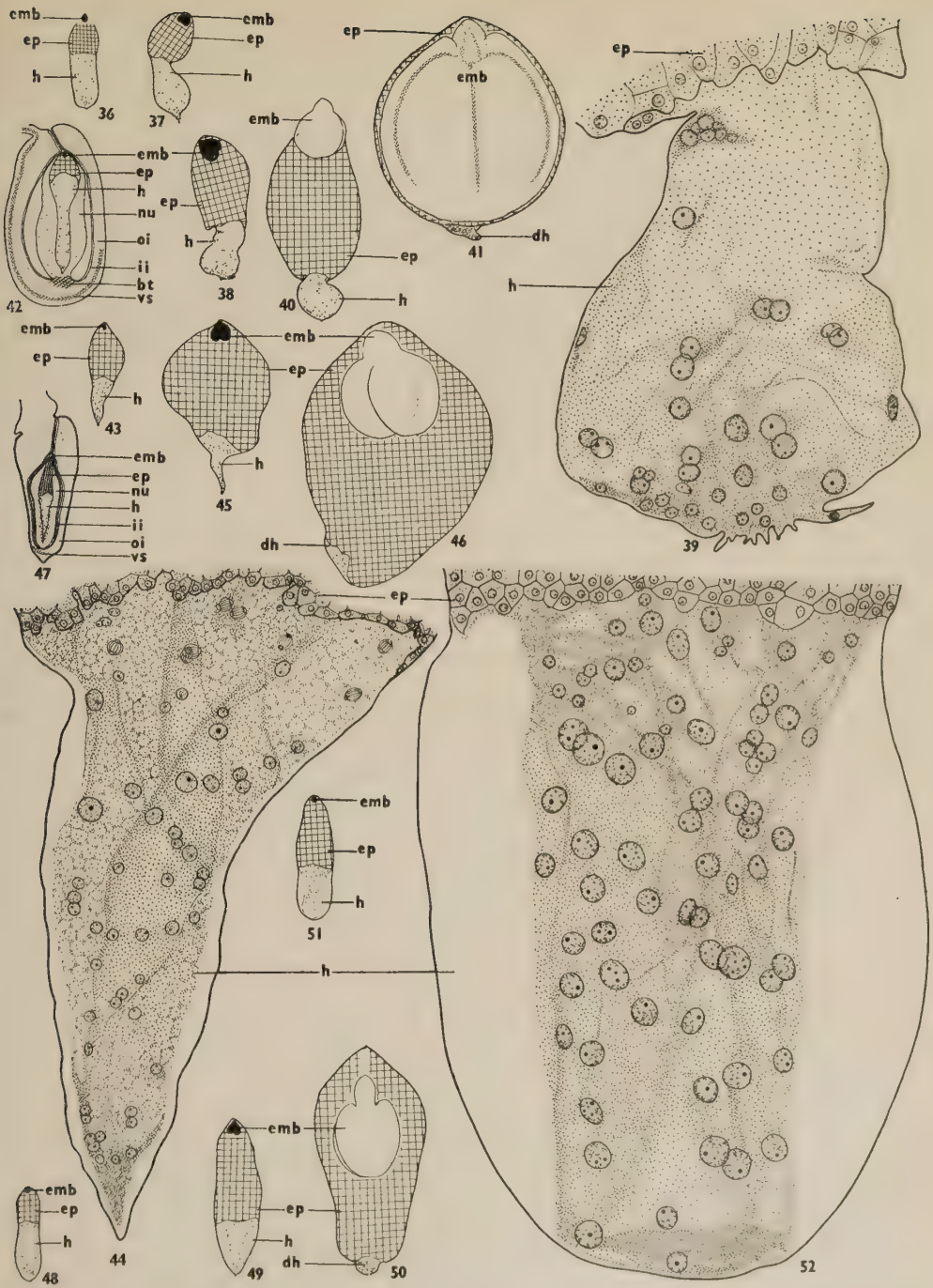
There is considerable variation in the genus *Desmodium*. The haustorium is free nuclear in *D. floribundum* and the narrow, tubular extension with the vesicular tip contains many peripherally distributed nuclei around a central vacuole (Figs. 22, 23). The thick-walled barrier tissue restricts its further growth (Fig. 23). *D. gangeticum* and *D. floribundum* are similar but in the former cell formation in



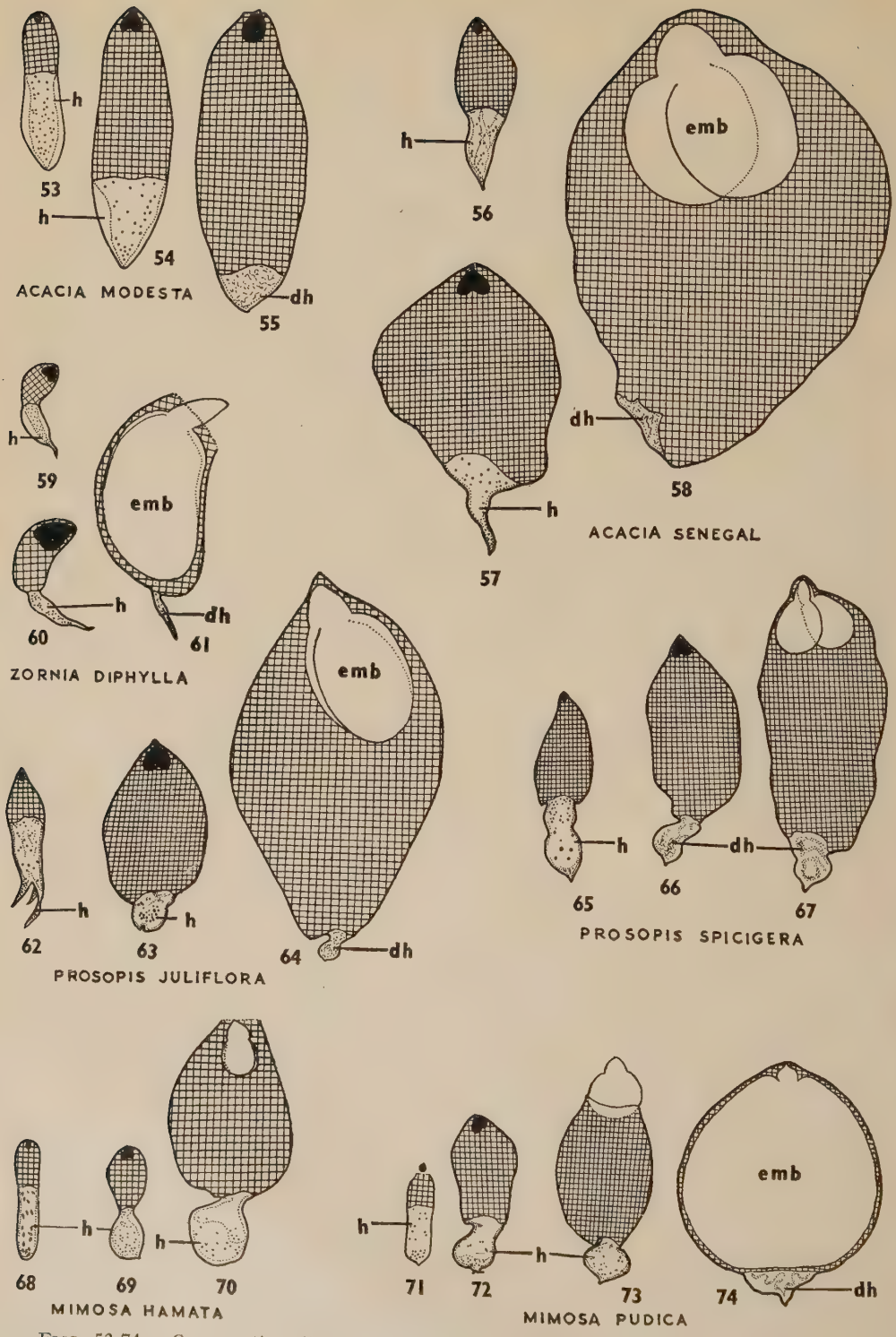
FIGS. 24, 25 — *Cassia sophera* (*emb*, embryo; *ep*, endosperm proper; *h*, haustorium). Fig. 24. Whole mount of endosperm at globular stage of the embryo (diagrammatic). × 28. Fig. 25. Enlargement of haustorium from Fig. 24; the nuclei are prominently spindle-shaped. × 220.



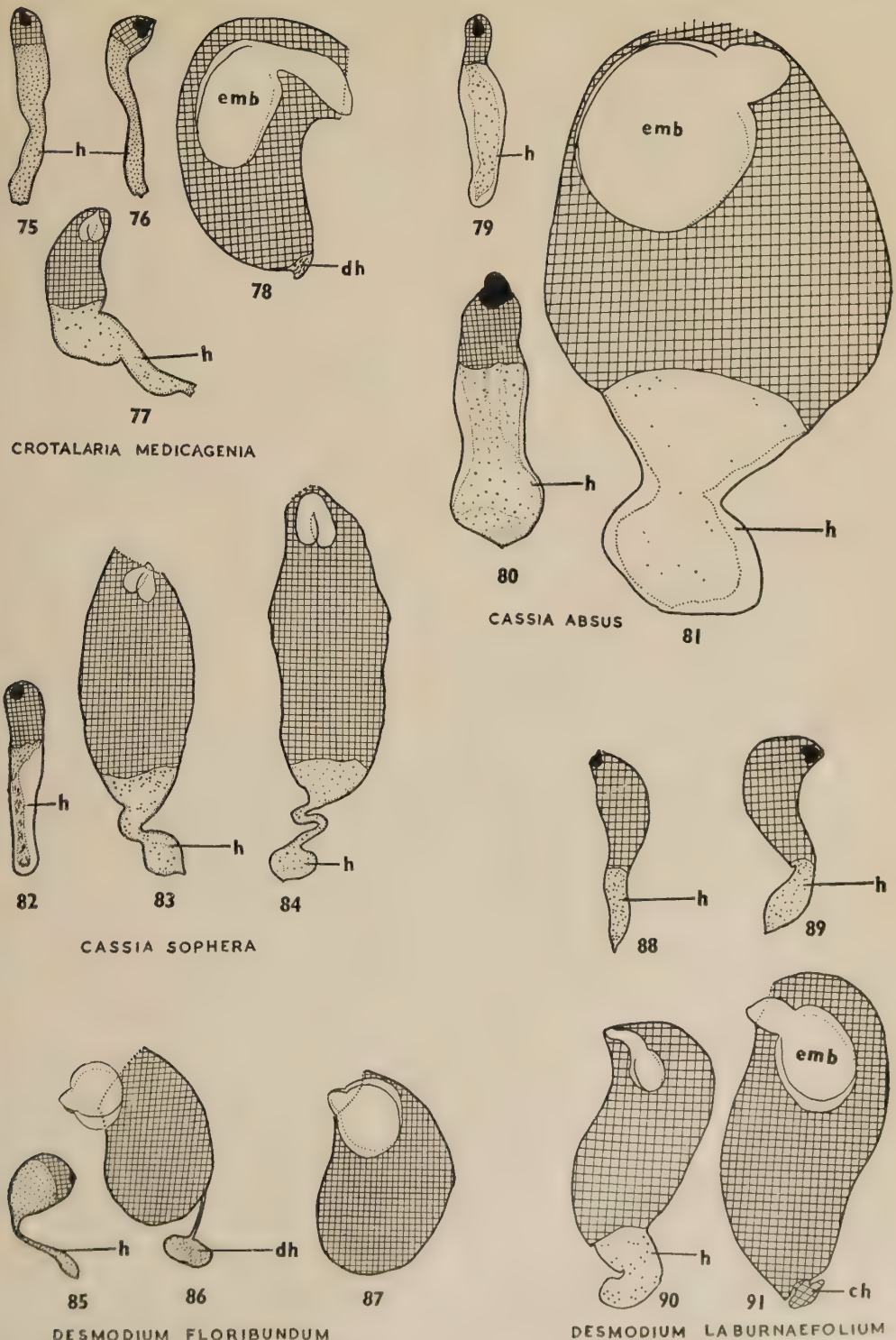
FIGS. 26-35 — *Prosopis*. Fig. 35 from microtome section, rest from whole mounts; all figures diagrammatic except Figs. 27, 29 and 32 (*bt*, barrier tissue; *dh*, degenerated remnants of haustorium; *dnu*, degenerated remnants of nucellus; *emb*, embryo; *ep*, endosperm proper; *h*, haustorium; *ii*, inner integument; *oi*, outer integument; *vs*, vascular supply). Figs. 26-30. *P. juliflora*. Figs. 26, 28, 30. Endosperm at different stages of the embryo. × 15. Figs. 27, 29. Enlarged views of haustorium from Figs. 26 and 28 respectively; note the peculiar finger-like processes at the tip of the haustorium in Fig. 27. × 183. Figs. 31-35. *P. spicigera*. Figs. 31, 33, 34. Same stages as in Figs. 26, 28, 30. × 15. Fig. 32. Magnified view of haustorium from Fig. 31. × 183. Fig. 35. L.s. young seed showing free nuclear bulbous tip of the haustorium. × 15.



FIGS. 36-52 — Figs. 42 and 47 from microtome sections, rest from whole mounts; all figures diagrammatic except Figs. 39, 44 and 52 (*bt*, barrier tissue; *dh*, degenerated remnants of haustorium; *emb*, embryo; *ep*, endosperm proper; *h*, haustorium; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument; *vs*, vascular supply). Figs. 36-42. *Mimosa pudica*. Figs. 36-38, 40, 41. Endosperm at different stages of the embryo. × 20. Fig. 39. Enlargement of haustorium from Fig. 38. × 160. Fig. 42. L.s. young seed at globular stage of the embryo. × 20. Figs. 43-47. *Acacia senegal*. Figs. 43, 45, 46. Same as Figs. 36, 38, 40. × 20. Fig. 44. Magnified view of haustorium from Fig. 43. × 160. Fig. 47. L.s. young seed. × 20. Figs. 48-50. *A. modesta*. Endosperm at different stages of the embryo. × 20. Figs. 51, 52. *Ulbrichia lehbek*. Fig. 51 Endosperm at globular stage of the embryo. × 20. Fig. 52. Haustorium magnified from Fig. 51. × 160.



FIGS. 53-74 — Comparative diagrams of whole mounts of the endosperm (*dh*, degenerated remnants of haustorium; *emb*, embryo; *h*, haustorium; the cross-hatched portion represents the endosperm proper). × 22.



FIGS. 75-91. — Comparative diagrams of whole mounts of the endosperm (*ch*, cellular haustorium; *dh*, degenerated remnants of haustorium; *emb*, embryo; *h*, haustorium; the cross-hatched portion represents the endosperm proper). × 22.

the endosperm extends only up to the tubular portion, while the vesicular tip remains free nuclear (Figs. 20, 21). In *D. laburnaefolium* at first the haustorium is free nuclear (Figs. 13-16), but later it becomes completely cellular (Fig. 17-19). At this time the peripheral cells bulge out prominently and they contain large multinucleolate nuclei with dense cytoplasm (Fig. 19). The cells of the endosperm proper in contact with the haustorium show a multinucleate condition (Fig. 15).

At the early globular stage of the embryo the haustorium of *Cassia sophera* is a tubular, sac-like structure similar to that of *Dalbergia*. During later stages the lower end becomes vesicular as in *Desmodium floribundum*. A feature of interest is the coiling of the tubular portion (Figs. 82-84). In one instance the nuclei had assumed a spindle-shaped appearance (Figs. 24, 25).

Figs. 53-91 represent the structure of the endosperm proper and the haustorium at the globular, heart-shaped and early dicotyledonous stages of the embryo. It may be noted that in most cases the haustorium occupies almost half the length of the endosperm (Figs. 53, 56, 59, 65, 68, 71, 80, 82, 88). The tip may be round (Figs. 53-55), tapering (Figs. 56, 57, 59, 60, 65, 66), or vesicular (Figs. 69-73, 80, 81). Sometimes the surface area of the haustorium is increased by the tip ending in ridges and furrows (Figs. 76, 77) or in finger-like processes containing dense cytoplasm and large nuclei (Fig. 62), or the tubular portion is coiled as seen in Figs. 83, 84.

Discussion

The family Leguminosae comprises 600 genera and 1200 species of which endosperm development has so far been studied only in about 30 genera and 60 species. All the 14 genera and 20 species investigated by us have a chalazal haustorium. This is characterized by the larger size of the nuclei; denser cytoplasm especially at the tip; and the gradual digestion of the adjacent nucellar tissue, sometimes including the integument.

Narasimhachar (1948) in *Acacia farnesiana*, and Anantaswamy Rau (1953, 1955) in some species of *Acacia*, *Crotalaria*, *Dalbergia*, *Desmodium*, *Mimosa* and *Zornia*, no doubt observed the chalazal free nuclear portion of the endosperm but did not attribute any haustorial role to it. This now seems clear except in *Acacia farnesiana*.

Pantulu (1951) recorded a hasutorium in *Cassia occidentalis*, *C. auriculata* and *C. glauca*, but his figures do not bring out the comparative stages of the embryo and the endosperm. For instance, his Fig. 4 shows only the haustorium but not the embryo while in Fig. 6 the embryo is shown but the distal part of the haustorium is missing.

Dnyansagar's (1951, 1954c) account of *Mimosa hamata* is misleading. He states: " In the course of time the whole of the endosperm becomes cellular and fills the cavity of the seed." This can be true only of the upper part of the endosperm. The lower half of the endosperm remains free nuclear, assumes a vesicular outline, and functions as a haustorium.

A cellular haustorium has been reported only in *Cyamopsis psoralioides* and *Desmodium pulchellum* (Anantaswamy Rau, 1953). *Desmodium laburnaefolium* may now be added to this list. Three species of *Desmodium* (*D. tortuosum* and *D. triflorum* — Anantaswamy Rau, 1953; *D. floribundum* — present work) show a free nuclear condition, while in *D. gangeticum* (present work) the haustorium is partly cellular and partly free nuclear.

Rothia trifoliata (Anantaswamy Rau, 1951b) develops a lateral endosperm haustorium on the funicular side, which can be compared to the ' Divertikel ' reported in some members of the Caryophyllaceae (Rocèn, 1927) and in *Macadamia ternifolia* of the Proteaceae (Kausik, 1938c).

In *Vicia faba*, besides a chalazal haustorium, Kastikova (1955) also reports a micropylar endosperm haustorium. This point is being reinvestigated by us.

Endosperm haustoria are common in many families showing Cellular endosperm, but there are only few with Nuclear endosperm which show such a condition: Simarubaceae (Wiger, 1935; Anantaswamy Rau, 1940), Proteaceae (Kausik, 1938a, b, c; 1939; 1942), Cucurbitaceae (Chopra, 1953, 1954, 1955;

Chopra & Agarwal, 1958; Weiling & Schagen, 1955; Johri & Roy Chowdhury, 1957; Singh, 1955, 1957), and Rutaceae (Johri & Ahuja, 1957). In the order Rosales, Leguminosae is the only family so far found to have endosperm haustoria.

Summary

The development and behaviour of the chalazal endosperm haustorium has been investigated in 8 genera and 10 species of the Lotoideae, 2 genera and 3 species of Caesalpinioideae, and 4 genera and 7 species of Mimosoideae.

The endosperm is of the Nuclear type and centripetal walls are laid down at the micropylar end even before the proembryo has reached the globular stage.

The cellular condition is usually restricted to the upper half of the embryo sac resulting in a massive tissue designated as the endosperm proper. The lower portion of the endosperm generally remains free nuclear and functions as an aggressive haustorium. In *Desmodium laburnaefolium* it becomes completely cellular but in *D. gangeticum* cell formation does not extend to the vesicular tip.

The size of the haustorium is very variable. It usually reaches up to the base of the ovule and its further extension is checked by the development of a thick-walled barrier tissue at the chalazal end.

The shape of the haustorium shows gradations from a uniformly broad sac-like structure merging with the endosperm proper as in *Albizzia, Alhagi, Argyrolobium, Delonix* and *Vigna* to a narrow, coiled and tubular structure with a dilated vesicular tip as in *Cassia sophera* and *Desmodium floribundum*. Intermediate forms occur in *Acacia senegal, Cassia absus, Crotalaria medicagenia, Mimosa hamata, M. pudica, Prosopis spicigera* and *Zornia diphylla*. In *Prosopis juliflora* the tip of the haustorium ends in several finger-like processes which penetrate into the tissue of the nucellus.

The haustorium contains dense cytoplasm with many free nuclei. It is functional up to the heart-shaped stage of the embryo after which its activity declines. In a few genera like *Desmodium* and *Tephrosia* it remains active till the cytoledons have become quite prominent.

The endosperm proper consists of uninucleate cells but close to the haustorium some of the cells may be multinucleate, e.g. in *Tephrosia villosa* and *Desmodium laburnaefolium*. The food reserve is mostly protein, but oil is present in *Alhagi camelorum, Cassia sophera, Dalbergia sissoo* and *Vigna vexillata*, and starch occurs in small quantities in *Delonix regia*.

As the endosperm proper fills the seed cavity, the haustorium shrinks and collapses, but its remnants persist up to the early dicotyledonous stage of the embryo. During its growth and maturation the embryo consumes the endosperm and only one or two layers persist in the seed.

We are grateful to Professor P. Maheshwari for comments and valuable suggestions. Thanks are also due to Drs R. N. Chopra and R. N. Kapil for their assistance in the preparation of this paper.

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I. THE EFFECTS OF LIGHT OF VARIOUS QUALITIES ON THE DEVELOPMENT OF THE PROTONEMA AND BUD FORMATION IN *POHLIA NUTANS* (HEDW.) LINDB.

G. C. MITRA*, A. ALLSOPP & P. F. WAREING**
Department of Cryptogamic Botany, Manchester University, Manchester, U.K.

Introduction

It has been known since the early work of Klebs (1893) that the development of the moss protonema is greatly affected by the light intensity. Klebs discovered that a relatively high light intensity is necessary for the initiation of leafy shoots on the protonema of *Funaria hygrometrica*, while lower light intensities are sufficient for the continued growth of the protonema. Later workers (e.g. Goebel, 1896; Servettaz, 1913; Ubisch, 1913; Robbins, 1918; Pringsheim & Pringsheim, 1935) also found that light is essential for the formation of shoot-buds. The single claim to the contrary (Keil, 1949) has not been confirmed and is possibly explained by the fact that minute bud primordia, initiated in light, can continue their development on protonema inocula transferred to suitable media in darkness (Fries, 1945).

Notwithstanding the evident importance of light, there have been few detailed studies of the effects of this factor on the morphology of the protonema or on the process of bud development. Most investigations on the effects of light on the development of representatives of the Bryales have been restricted to studies of the role of light in spore germination (*see* Meyer, 1948; Fitting, 1950). Even in this field only isolated workers have made use of light of different wavelengths. Listowski (1927) followed spore germination and growth of the protonema of a number of moss species under white, red, blue and green light, but he gave no details of the spectral limits of his light sources. Stephan (1928) used light of defined spectral limits, but his observations were restricted to spore germination and the early stages of protonemal growth. Reference is made below to the investigations of Pringsheim & Pringsheim (1935) on the effects of different colours of light on protonemal growth and bud formation and to some observations by Wettstein (1953) on the effects of red light on the protonema of *Funaria*.

The necessity for further investigation of the effects of the light factor was emphasized by the claim of Sironval (1947) that in the development of the protonema of *Funaria hygrometrica* two phases may be recognized, namely, *chloronema* and *caulonema*, of which only the latter is capable of giving rise to buds of leafy shoots. Later work by Allsopp and Mitra (1956, 1958) disproved the contention of Sironval that shoot-buds are never produced in sterile cultures in absence of natural daylight, although, in agreement with Sironval and subsequent workers (Bopp, 1952, 1954, 1955, 1957; Kofler, 1956), an increased differentiation of protonemal filaments was observed in older cultures. The present investigation was undertaken to determine the possible role of different spectral regions in the differentiation of the protonema and in the formation of the buds of leafy shoots on the latter.

Material and Methods

In contrast with the previous work (Allsopp & Mitra, 1956, 1958) in which

*Present Address: National Botanic Gardens, Lucknow, India. This investigation was carried out during the tenure of a Fellowship under the Colombo Plan.
**Department of Botany, University College of Wales, Aberystwyth, U.K.

the development of the protonema was followed in species of 14 genera of the Bryales, in the present work, because of limitations in space available in the various light chambers, only one species was investigated, namely *Pohlia nutans* (Hedw.) Lindb. Since ripe spores of this or other suitable species were not available at the beginning of the present investigation, the experiments were started from a stock protonema culture. It had been found in the previous work that apart from the very earliest stages of growth, the development of a culture from a small protonema inoculum was virtually identical with that from one or a group of spores.

As in earlier work, the basic medium was a half-strength Knop's solution, with addition of Berthelot's (1934) mixture of trace elements. Except in the series of liquid cultures, washed agar was added to give a final concentration of 1 per cent. In the treatments, using the various light sources, two parallel series of cultures were prepared, one with, and one without, the addition of 1 per cent sucrose. In all experiments a parallel series of cultures were grown in daylight of a north window of the laboratory to ensure that normal development of the protonema could take place when suitable conditions were supplied. The main series of cultures were grown in Petri dishes, containing 30 ml of the medium. In some experiments an additional series of 100 ml Erlenmeyer flasks was used, each with 50 ml of medium. Liquid cultures were prepared in large test tubes containing 30 ml of medium. All experimental treatments were carried out in triplicate.

Light Sources — The light sources consisted of coloured fluorescent tubes used in conjunction with coloured "Perspex" as filters. Details of the four light sources are summarized in Table 1.

The approximate spectral distribution of each source was determined from the published data on the emission of fluorescent tubes (Withrow & Withrow, 1956) and of the transmission properties of the "Perspex" (King & Ventura, 1951). The infra-red source consisted of a 150 watt tungsten filament lamp, used together with an Ilford infra-red filter (207) and a 9·5 cm thick water screen. The radiation from this source was calculated to lie within the range 730-1,000 mμ.

TABLE 1

Fluorescent source	Filter	Approximate spectral distribution
Red	Red "Perspex" (400)	580-700 mμ
Green	Green ,, (600)	460-600 mμ
Blue	Blue ,, (705) + 1 cm. cell of M/3 copper chloride	400-530 mμ
White	—	—

The equipment was arranged so that the irradiation intensity at the level of the cultures was effectively same (200 μW/cm²) for each source. The energy levels were determined by means of a barrier-layer cell which had been calibrated, for each source, against a thermopile. During each 24-hour period the cultures were illuminated for 18 hours. The temperature was thermostatically controlled at 22°-26°C.

Experimental Results

Effects of Different Light Sources — Two experiments were carried out on the effects of various light sources. In the first experimental series, the cultures were exposed to the visible light sources only. In the second, one set of cultures from each visible light source received an additional exposure of 2 hours to the infra-red radiation. The results of the two series of experiments will be considered together.

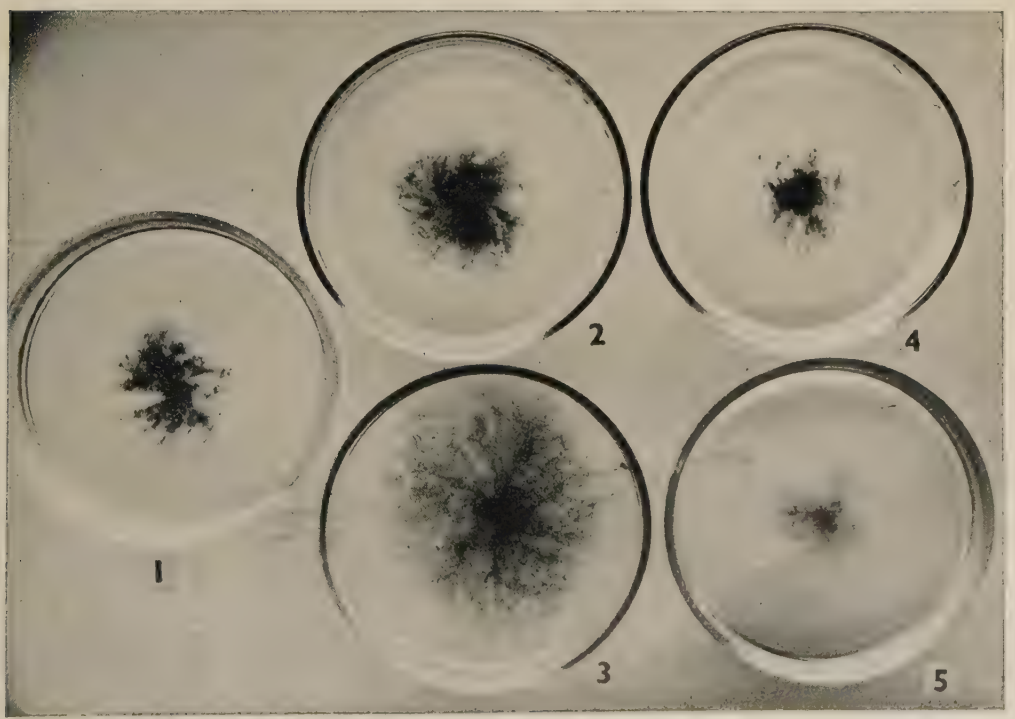
For the first two weeks or so after inoculation, until the cultures became well-established, there was little difference between the cultures, but with further exposure differences became increasingly marked. Some of the effects of the various light sources on the general growth of the cultures are summarized in Table 2 and illustrated by Figs. 1-5.

It is evident from Table 2 and Figs. 1-5 that maximum horizontal spread of the cultures occurred in blue light. Minimum spread was obtained in green light, in which the cultures were very

TABLE 2 — EFFECTS OF DIFFERENT LIGHT SOURCES ON THE RADIAL GROWTH OF THE PROTONEMA AND BUD DEVELOPMENT

	Mean diameter of cultures in mm*					Mean number of buds per culture			
Date†	3-4-57	11-4-57	17-4-57	29-4-57	6-5-57	11-4-57	17-4-57	29-4-57	6-5-57
(a) *Without sucrose*									
Daylight	11	19	21	28	34	6	21	32	43
White light	20	25	28	37	47	0	0	1	10
Red light	12	15	20	25	29	0	0	2	4
Blue light	24	37	44	59	63	0	0	0	0
Green light	6	12	13	17	27	0	0	0	0
(b) *With* 1% *sucrose*									
Daylight	14	22	27	38	40	4	32	73	102
White light	23	29	33	41	55	0	0	1	2
Red light	19	25	30	37	45	0	0	19	23
Blue light	29	43	51	67	73	0	0	0	0
Green light	7	14	24	24	28	0	0	0	0

*Mean of measurements or counts of three similar (Petri dish) cultures.
†Cultures started on 12-3-57.



FIGS. 1-5 — *Pohlia nutans*. Effects of light of various qualities on the development of the protonema and bud formation (without sugar). Fig. 1. Daylight. Fig. 2. White artificial light. Fig. 3. Blue light. Fig. 4. Red light. Fig. 5. Green light. — All Figs. × ½.

attenuated. Healthy looking cultures were produced in red light, and as found previously, in daylight and in white artificial light. It is also evident that under the particular experimental conditions the addition of 1 per cent sucrose to the medium had some effect on the growth of the cultures.

The first shoot-buds appeared in daylight cultures after about four weeks of growth, but did not appear in any of the artificial light cultures until approximately a further fortnight had elapsed. Buds were then observed in cultures in red light and in white light, but not in blue or green light. Even after a further exposure of many weeks, no trace of shoot-buds was observed in the cultures from blue or green light. Bud formation was very active in red light, especially in the presence of sucrose. Only organized buds were counted, but microscopic examination revealed numerous globular swellings on the protonema, suggesting that many buds had been initiated but failed to develop further. The earlier formation and greater number of buds in daylight cultures does not imply any superiority of daylight over white or red artificial light in this respect, since the intensity of daylight was uncontrolled. Furthermore, our previous work had shown that in strong white artificial light as many shoot-buds may be formed as in daylight. The results do imply, however, that the relatively low artificial light intensity employed in the present work approached the critical level for the formation of shoot-buds. It is also clear that, under the particular experimental conditions at least, blue and green lights are less favourable for bud formation than red light or white light. Notwithstanding the good protonemal growth in blue light, there was no trace of bud formation, and it may be doubted whether shoot-buds would be produced in blue or green light of greater intensity.

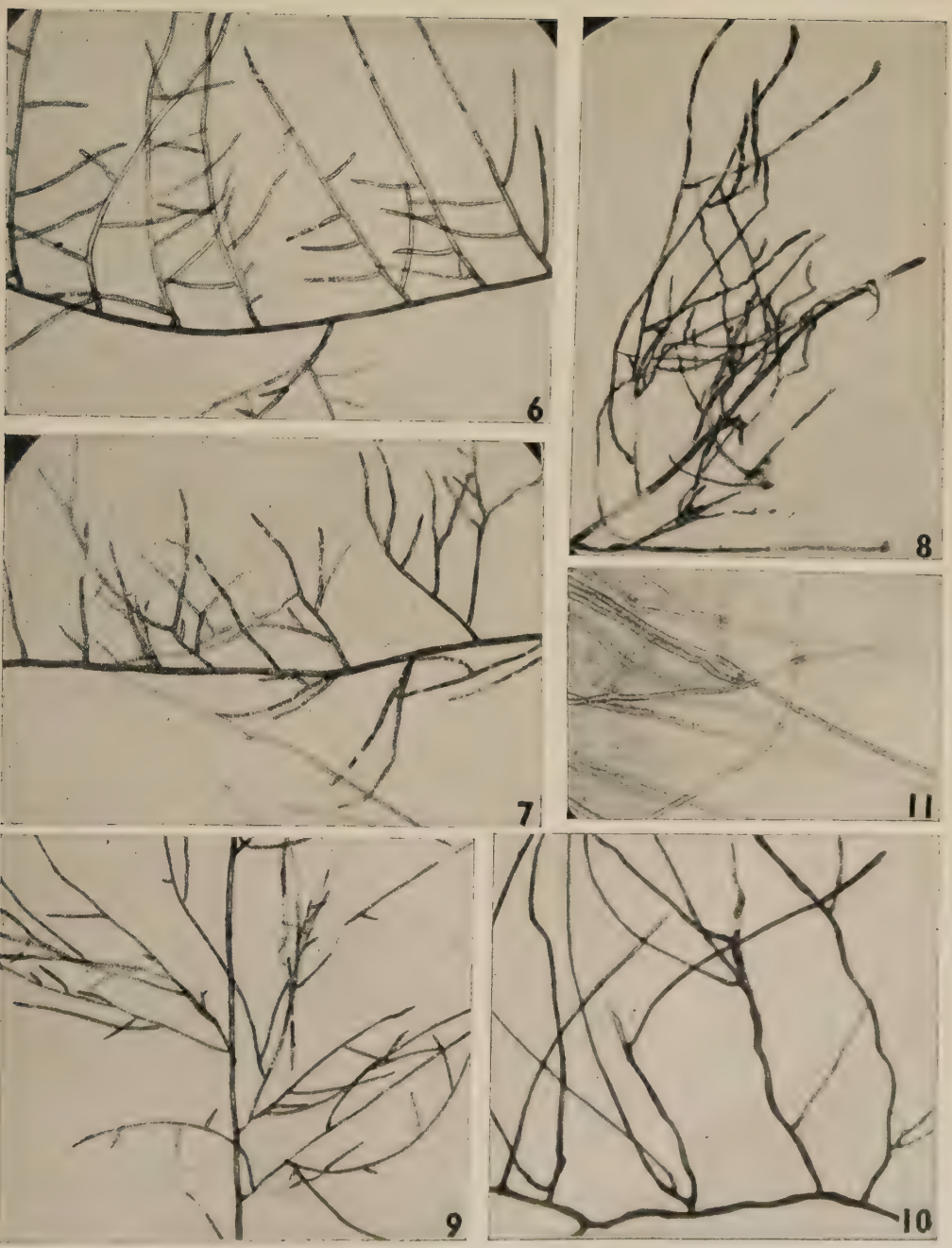
It seems likely that the formation of shoot-buds results in a check to protonemal growth, for in daylight, in which a large number of strongly growing shoots were produced, the protonema was less in surface area than in white artificial light, where fewer buds were formed. The protonema was also of restricted extent in the red light, while in blue light, in which no buds were formed, the protonema showed maximum extension. It is also shown in Table 2 that sucrose had relatively little effect on protonemal growth or bud formation in the blue light.

All the cultures with sucrose were somewhat darker than those without it, due to an increase in the brown-pigment of the cell-walls. They were also somewhat less compact in appearance, owing to a rather more marked development of the relatively main axes of the protonema.

After approximately two months of growth, a detailed morphological observation of the cultures was made. The results of the examination of the first batch of cultures were confirmed by that of the second experimental series, and later by that of the flask cultures. The results obtained with the various light sources are treated separately.

Daylight and White Artificial Light — In daylight and white artificial light, both with and without sucrose, the protonema had a typical heterotrichous habit, with prostrate brown-walled filaments, showing oblique cross-walls and relatively few elongated chloroplasts, and erect aerial filaments with paler cell-walls, perpendicular cross-walls, and numerous round chloroplasts (Figs. 6, 7). Typical protonema-rhizoids were present.

Red Light — In red light a strong, healthy-looking protonema was produced. The heterotrichous habit was well shown, but the aerial system was relatively more developed than in white light, and occurred as dense tufts of erect branches. A characteristic feature of the cultures grown in red light was the occurrence of prominent nutation patterns in both prostrate and erect filaments (Fig. 8). Wettstein (1953), who investigated the effect of red light on early stages of the protonema of *Funaria*, also observed nutation patterns, but made no mention of similar curvatures in the rhizoids. In our cultures certain very peculiar rhizoids occurred, which coiled tightly like tendrils around the much thicker parent filaments. Further, the tips of the aerial filaments were often noticeably distended. In older cultures, normal shoot-buds were



FIGS. 6-11 — *Pohlia nutans*. Effects of light of various qualities on the morphology of the protonema. Fig. 6. Daylight. Fig. 7. White artificial light. Fig. 8. Red light. Fig. 9. Blue light. Fig. 10. Green light. Fig. 11. Complete darkness. Figs. 6-10. × 46 and Fig. 11. × 84.

present and in addition there were numerous rounded swellings, which, on further growth, had given rise to typical protonemal filaments.

Blue Light — The protonema was well-developed with a marked differentiation between strong, brown-walled filaments, with oblique cross-walls and few elongated chloroplasts, and their thinner branches, with hyaline cell-walls, perpendicular cross-walls and bright-green, rounded chloroplasts (Fig. 9). The latter filaments were not developed as a prominent erect system, but often ran horizontally or obliquely in or above the medium. These green filaments had somewhat elongated cells and their branches were often some distance behind the apical cross-wall. Pale protonema-rhizoids also occurred, but there was no indication of the formation of buds or other special structures.

Green Light — In green light growth of the protonema was very poor from the outset. The filaments became increasingly attenuated and there was no distinction in cellular characteristics between the relatively main filaments and their lateral branches (Fig. 10). There was thus no indication of a heterotrichous habit. The branches were few but arose in the normal position near an acroscopic cross-wall. They grew horizontally in the medium and showed no response to the direction of the light source. Some of the cells were swollen in an irregular, abnormal fashion. Buds and other specialized structures were absent. The whole protonema was yellowish green as a result of scanty development of chloroplasts in the majority of the filaments. It is evident that green light is unsuitable for either growth of the protonema or the formation of shoot-buds.

Infra-red Radiation — The exposure to an additional two hours of infra-red radiation had no effect on either the morphology of the protonema or the formation of buds in cultures from any of the light sources.

Other cultures, growing on a 1 per cent sucrose medium, were exposed to infra-red radiation only, either continuously or for two hours, followed by the remainder of the 24-hour period in darkness. These cultures were in no way different from normal dark cultures (*see* below). When sugar was omitted from the medium, there was virtually no growth in the cultures exposed to the infra-red radiation only.

Dark Cultures — Cultures were prepared on the normal agar medium, with addition of 0·5, 1·0, 2·0 or 4·0 per cent sucrose. There was no growth in the absence of sugar, but in the cultures supplied with sugar the protonema spread slowly but continuously over the medium. Growth was better at the three higher sugar-concentrations than on the 0·5 per cent medium. The main filaments and branches were almost invariably horizontal, only isolated aerial branches occurring. There was virtually no distinction between the main filaments and branches, which were very few (Fig. 11). All the filaments were slender with elongated cells. The latter had well-developed plastids, which were usually colourless but occasionally pale green. No traces of bud formation were seen in any of the dark cultures.

Discussion

The present investigation has demonstrated that the morphological pattern of the protonema of *Pohlia nutans* is remarkably stable even in cultures grown under light of different wavelengths. In all the visible light sources, with the sole exception of green light, the protonema showed the marked distinction between principal filaments of considerable length, and lateral branches of limited growth, described previously by Allsopp & Mitra (1956, 1958) for a range of species of the Bryales. In opposition to the views of Sironval (1947) no constant morphological difference could be established between the cultures from white artificial light and daylight. Furthermore, shoot-buds are formed as readily in artificial light as in natural daylight.

Although the protonema cultures from the various light sources showed many morphological similarities, it would seem that white light is essential for the development of a morphologically completely normal protonema. The differences ob-

served between protonema cultures from white light and from the other light sources might conceivably arise either as a consequence of (a) differences in photosynthetic efficiency in the different spectral regions, or (b) more specific formative effects of light. The work of several authors (e.g. Hoover, 1937) indicates that the action spectrum for photosynthesis contains peaks in the red and blue regions, with reduced efficiency in the green. This relationship suggests that in the present work the active growth of the cultures in red and blue light, and the attenuated growth in green light, might be largely accounted for by differences in photosynthetic efficiency. Nevertheless, it seems unlikely that the differences with respect to bud development can be explained solely in this way. Thus, although the cultures under blue light had the greatest diameter, indicating very active growth, no buds were formed even when sucrose was supplied in the medium. On the other hand, in red light there was less growth of the protonema, but abundant bud formation, especially when sucrose was supplied. Similar results had been obtained previously by Pringsheim & Pringsheim (1935), who observed in *Funaria* cultures that shoot-buds were formed on the protonema in white or red light, but not in blue or green. They also observed that protonema growth was fairly good in blue light, but that in green light the protonema consisted of little-branched, pale green filaments which closely resembled those produced in darkness. The results of the present investigation confirm and extend those of Pringsheim & Pringsheim (1935) and would appear to indicate that there is a specific formative effect of red light, or, less probably, an inhibiting effect of blue light on bud formation. In this connection it may be mentioned that although the present work and that of previous authors (Servettaz, 1913; Ubisch, 1913; Robbins, 1918; Pringsheim & Pringsheim, 1935) has shown that moss protonema can be grown for an apparently indefinite period in total darkness on various culture media, growth is usually very slow and there is no convincing evidence of the production of shoot-buds on protonema cultured entirely in the dark. This evidence suggests that specific substances, formed only in the light, may be necessary for normal growth of the protonema and bud formation, although there is also the possibility that growth of the protonema on organic media may be restricted by the permeability and nutritional value of the organic substances hitherto supplied.

The contrasting effects of red and blue light on moss protonema cultures are comparable to results obtained for many plant species with respect to internode extension, flowering and other responses (e.g. Wassink & Stolwijk, 1956; Van der Veen, 1958). In flowering plants, infra-red radiation is known to have marked effects on various light responses, frequently acting antagonistically to the influence of red light (Borthwick, Hendricks & Parker, 1952), but in the present work infra-red radiation had no evident effect.

In his recent investigations on the effects of mono-chromatic light of various wavelengths on the development of the "protonema", i.e. the filamentous stage of the gametophyte, of the fern *Dryopteris filix-mas*, Mohr (1956a, b) also found that radiation between 760 and 1,100 mμ had no effect on the "protonema" itself, although there was some inhibition of spore germination. In red light the filaments were much elongated and frequently showed nutation patterns.

It may be concluded from the present work that the protonema of *Pohlia nutans* is very sensitive to the action of light, and like many other plant materials shows a differential response to light of different spectral regions. The results suggest that normal protonemal development, and in particular the formation of buds of leafy shoots, may be dependent on specific formative effects of light, but further work will be necessary before this question is finally settled.

Summary

An account is given of the effects of light of different spectral regions on the development and morphology of the protonema, including the origin of shoot-buds, in *Pohlia nutans*. The protonema

was grown under aseptic conditions, on media both with and without the addition of sucrose. The growth of the protonema in total darkness was also followed.

In blue and red light, as in natural daylight and white artificial light, the protonema showed the normal differentiation into long filaments of unlimited growth and lateral branches of restricted development. No such differentiation was observed in green light or in darkness. Certain peculiarities, particularly nutation patterns, were observed in red light. Radial horizontal expansion of the protonema was greatest in blue light.

Buds of leafy shoots were formed readily in daylight, white artificial light and in red light, but never appeared in blue or green light, in darkness, or in continuous infra-red radiation, even when sucrose was supplied in the medium. The results suggest that red light has a specific formative effect on the initiation of shoot-buds on the protonema.

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II. THE EFFECTS OF SUGAR CONCENTRATION ON THE DEVELOPMENT OF THE PROTONEMA AND BUD FORMATION IN *POHLIA NUTANS* (HEDW.) LINDB.

G. C. MITRA* & A. ALLSOPP

Department of Cryptogamic Botany, Manchester University, Manchester, U.K.

In the previous contribution on the development of the protonema and the formation of buds in *Pohlia nutans* (Mitra *et al.*, 1959), some difficulty was encountered in making a distinction between general nutritive (trophic) effects and specific formative effects of light. The studies of several workers (e.g. Servettaz, 1913; Ubisch, 1913; Robbins, 1918; Pringsheim & Pringsheim, 1935) had already shown that moss protonemata, when maintained in darkness or under feeble lighting conditions, can continue to grow at the expense of sugar in the medium. Keil (1949) reported the formation of buds in complete darkness on protonema of species of *Splachnum*, growing on a 2 per cent sucrose medium. It, therefore, seemed not unlikely that a study of the effects of various concentrations of sugar might contribute towards a better understanding of the role of carbohydrate nutrition, and hence of photosynthesis, in the development of the protonema and bud formation in *Pohlia nutans*.

Material and Methods

The culture methods were similar to those adopted in previous investigations (*see* Allsopp & Mitra, 1956, 1958; Mitra *et al.*, 1959). In all experiments Knop's solution was used as the basic medium. The sugar concentrations employed are detailed in the accounts of individual experiments. Cultures were maintained in natural daylight at laboratory temperature, as well as in the temperature regulated culture room (22°-24°C.) with artificial lighting supplied by two fluorescent tubes ('Natural') giving a light intensity of approximately 150 foot candles at the level of the cultures. The light was switched on automatically for 16 hours and off for 8 hours. Agar cultures in Petri dishes, tubes and flasks, and liquid

*Present Address: National Botanic Gardens, Lucknow, India. This investigation was carried out during the tenure of a Fellowship under the Colombo Plan.

cultures in tubes were prepared from small protonema inocula of *Pohlia nutans*.

Experimental Results

The Effects of Sucrose Concentration — Parallel sets of three cultures per concentration were set up using the basic medium without sugar and with the addition of sucrose to give concentrations of 0·5, 1·0, 2·0 and 4·0 per cent.

After 38 days, in every culture, the protonema covered the whole surface of the agar medium. The protonema was markedly heterotrichous, although in the cultures containing 4 per cent sucrose, the erect system was feebly developed.

The effect of sucrose concentration on bud development is summarized in Fig. 1. During early stages of protonema growth (i.e. after 31 and 38 days) addition of 1·0 and 2·0 per cent sucrose to the medium led to an increase in the number of shoot-buds, although the first buds appeared at approximately the same time in cultures containing 0·0, or 0·5 per cent sucrose. The presence of 4 per cent sucrose, however, resulted in a marked depression of bud formation. In later stages of growth (47 days) even the lower concentrations of sucrose were somewhat inhibitory to bud formation.

Measurements of leafy plants of approximately equal age from older cultures (10 weeks) revealed a progressive reduction in length with increasing sucrose concentration (*see* Table 1). This reduction in length was largely due to a decrease in the length of the individual internodes. The size of the leaves also decreased with increasing sucrose concentration. Similar but more marked results were obtained with glucose concentrations.

The Effects of Glucose Concentration — Five concentrations of glucose (0·0, 0·5, 1·0, 2·0 and 4·0 per cent) have been tested. Parallel sets of three cultures per concentration were grown in natural daylight at laboratory temperature, and in artificial light at regulated temperature in the culture room.

Buds appeared at almost the same time in agar cultures without sugar and in those supplied with 0·5 or 1 per cent glucose. The effects of the different concentrations of glucose on the number of buds produced on the protonema are shown graphically in Fig. 2. The addition of 1 per cent glucose increased the rate of bud formation during early stages of protonemal growth, but subsequently the presence of glucose (even at low concentrations) was somewhat inhibitory to the further production of buds. At higher concentrations, the inhibitory effect of glucose was very marked, even in early stages of protonemal growth. Under both daylight and artificial light, very few buds were formed on agar cultures containing 4 per cent glucose, while in the parallel series of liquid cultures bud formation in 4 per cent glucose media was completely inhibited in artificial light, although a few buds were formed in daylight (Fig. 3). Buds appeared much earlier and in greater number in the daylight than in the artificial light cultures, while protonemal growth was greater in the latter. This

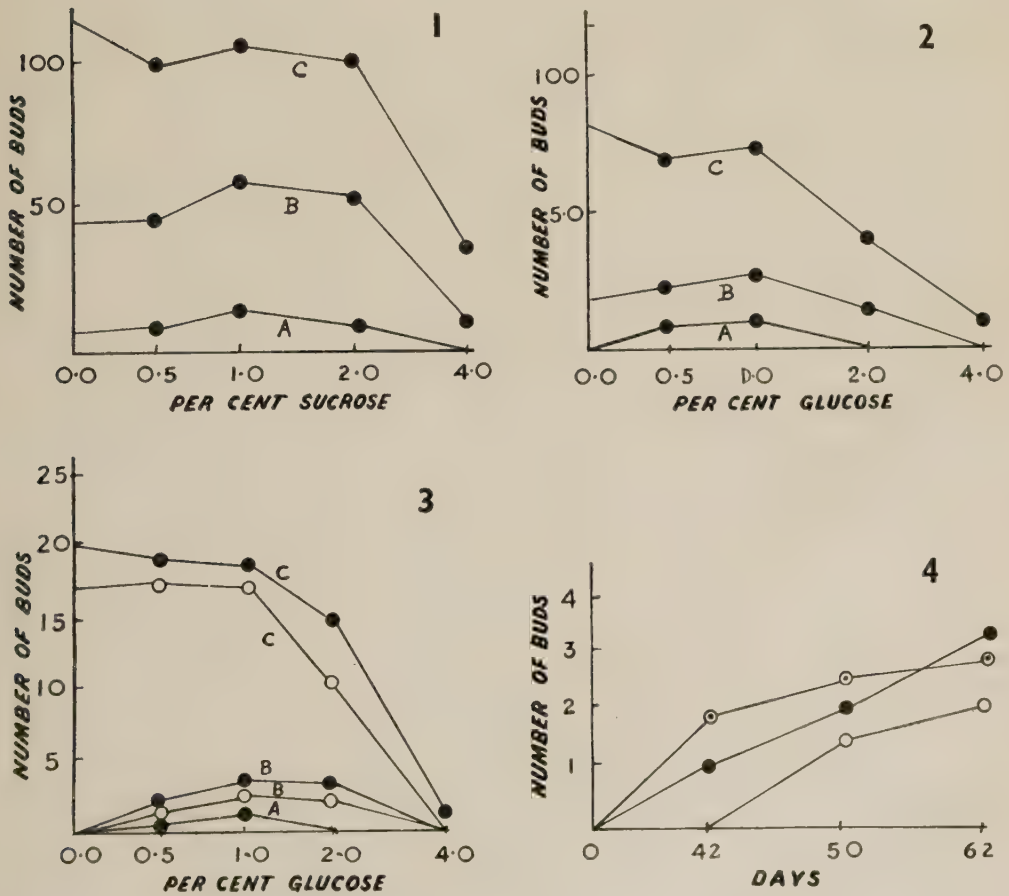
TABLE 1 — EFFECTS OF SUCROSE CONCENTRATIONS ON LEAFY PLANTS OF *POHLIA NUTANS**

Sucrose concentration in %	Height of plant in mm		Internode length in mm		Leaf length in mm	
	Range	Mean	Range	Mean	Range	Mean
0·0	7·7-18·0	11·9	0·32-0·72	0·53	0·65-1·20	0·86
0·5	5·9-9·5	7·7	0·18-0·54	0·38	0·32-0·77	0·55
1·0	5·9-9·0	6·9	0·23-0·63	0·34	0·27-0·59	0·42
2·0	4·7-9·0	6·6	0·14-0·45	0·24	0·23-0·54	0·37
4·0	3·6-7·4	5·0	0·14-0·27	0·19	0·18-0·32	0·24

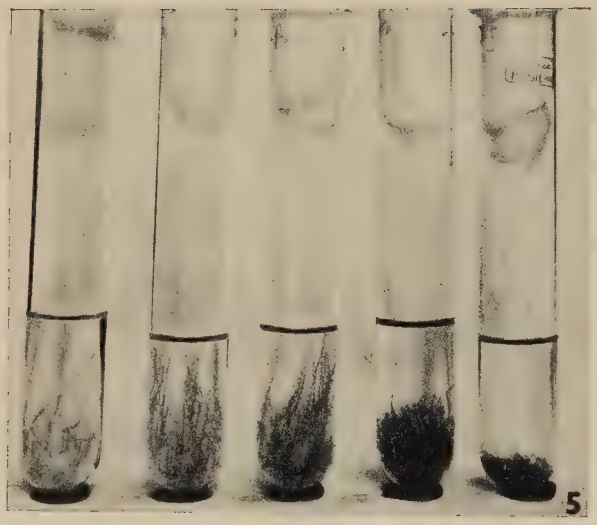
*Based on mean of 20 readings at random.

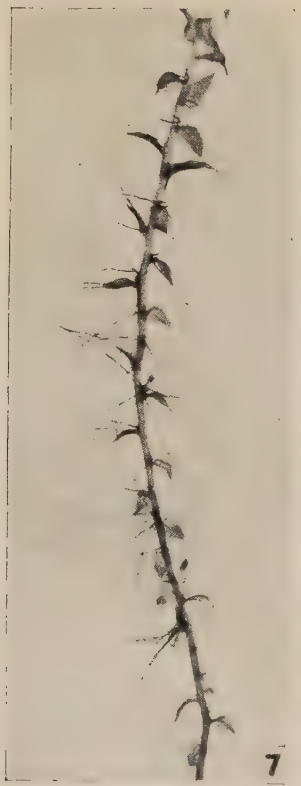
reciprocal relationship between protonemal growth and bud formation is similar to that observed in the previous investigation on the effects of light of various qualities on protonema development (Mitra *et al.*, 1959). It should be pointed out, however, that the cultures in daylight and in artificial light are not strictly comparable, since the light intensity and temperature were uncontrolled in the daylight cultures.

In addition to its effects on protonema growth and bud formation, the glucose concentration had a pronounced effect on further development of the leafy shoots (Fig. 5). Figs. 6-9 illustrate typical leafy shoots from liquid cultures grown in daylight, and similar results were also obtained with agar cultures from both daylight and artificial light, and in liquid cultures from artificial light.



FIGS. 1-4 — *Pohlia nutans*. Effects of various media on bud formation on the protonema. Mean values from three parallel series of cultures. Fig. 1. Effect of sucrose concentration. Agar cultures in artificial white light: *A*, after 31 days; *B*, after 38 days; *C*, after 47 days. Fig. 2. Effect of glucose concentration. Agar cultures in artificial white light: *A*, after 30 days; *B*, after 37 days; *C*, after 48 days. Fig. 3. Effect of glucose concentration. Liquid cultures: —●—●—, daylight; —○—○—, artificial white light. *A*, after 30 days (no bud formation in artificial white light); *B*, after 37 days; *C*, after 48 days. Fig 4. Effects of glucose, mannitol, and glucose + mannitol. Liquid cultures under artificial white light: ⊙—⊙—, 2 per cent glucose; ●—●—, 1 per cent glucose + 1 per cent mannitol; ○—○—, 2 per cent mannitol.





FIGS. 5-9 — *Pohlia nutans*. Effects of glucose concentration on protonema growth and shoot structure. Liquid culture in daylight. Fig. 5. Left to right, cultures in 0·0, 0·5, 1·0, 2·0 and 4·0 per cent glucose media. × ½ natural size. Figs. 6-9. Leafy shoots from 0·0, 1·0, 2·0 and 4·0 per cent glucose media respectively. × 5.

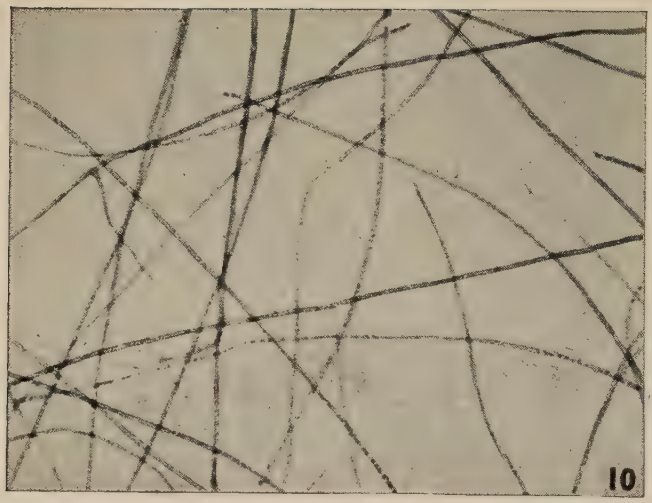
Optimal growth of the leafy shoots is obtained in 0·5 and 1·0 per cent glucose (Figs. 5, 7). At these concentrations, the plants have thicker and longer internodes and larger leaves than the attenuated plants from the control mineral medium. With 2 and 4 per cent glucose, there is a pronounced and progressive decrease in shoot height, in the length of the internodes and in the size of the leaves, the shoots from 4 per cent glucose being greatly stunted. In liquid cultures there is often a prominent development of rhizoids on the leafy shoot, whereas in agar cultures, rhizoids are restricted to the base of the aerial shoot.

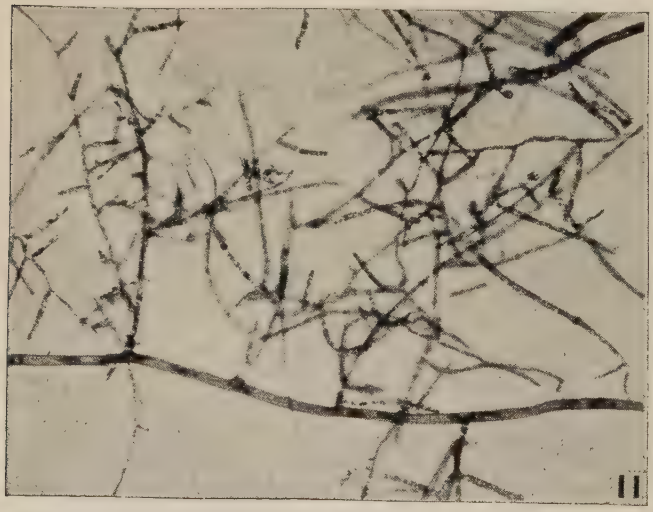
Different concentrations of sugar and the solid or liquid nature of the medium also affected the morphology of the protonema. On agar cultures the protonema developed its heterotrichous habit. In liquid cultures also there was usually a fairly marked distinction between the principal, little-branched, filaments and the short, richly-branched, laterals (Figs. 11, 12). In the absence of sugar, however, protonemal filaments were very attenuated and little-branched (Fig. 10). In fact, apart from their green colour, they were almost similar to the filaments previously observed in cultures grown in darkness. Protonemal development was also somewhat abnormal in the 4 per cent glucose media. At this concentration, although, in daylight, there was still a differentiation between the main and lateral filaments, the cells of the main filaments were thinner and paler, while those of the erect filaments were of more irregular shape than in the protonema from the lower sugar concentration (Fig. 12). In the parallel cultures from artificial light, scarcely any distinction was observed between the main and lateral filaments. In the cultures on the 4 per cent glucose media, the protonemal filaments showed characteristic nutation pattern; in artificial light all the filaments but in daylight only the filaments of restricted growth were affected. Swollen and irregularly shaped cells were also observed in 4 per cent glucose cultures, more frequently in daylight cultures. These cells might represent abortive attempts at bud formation. As in previous investigations, all cultures on media containing sugar were of darker appearance than the cultures on mineral media on account of an increase in the brown pigmentation of the cell walls.

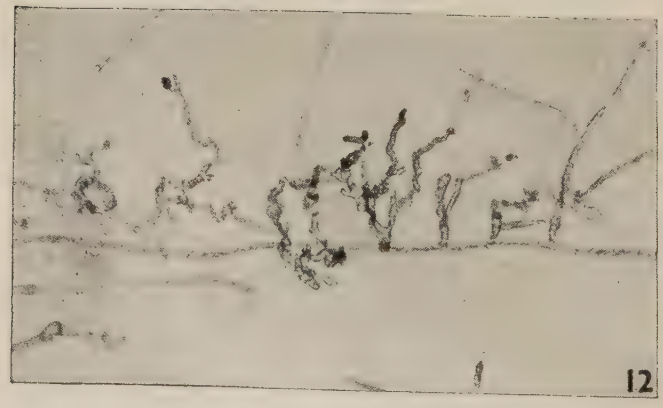
The Effects of Mannitol — The effects of sugar concentration described in previous sections might conceivably result from either nutritional or osmotic changes in the culture medium, or perhaps both of them might be involved. In an attempt to draw a distinction between the nutritional and osmotic effects of glucose concentration, a series of experiments were carried out in which the osmotic concentration of the glucose medium was increased by the addition of mannitol, which, as shown in control cultures, is nutritionally inert to *Pohlia*.

Parallel sets of three cultures per concentration were prepared on liquid media, consisting of the basic medium only, and basic medium with the following additions: 1 per cent mannitol; 1 per cent glucose + 1 per cent mannitol; 2 per cent glucose; 2 per cent mannitol; 1 per cent glucose + 3 per cent mannitol; 4 per cent glucose; and 4 per cent mannitol. The cultures were grown in the artificial light and at the regulated temperature.

No buds were produced in 4 per cent mannitol, 1 per cent glucose + 3 per cent mannitol, and 4 per cent glucose media. The results summarized in Fig. 4 indicate that the effects of glucose at lower concentrations, 1 and 2 per cent, are not entirely of osmotic origin, since raising the osmotic concentration by the addition of mannitol resulted in some inhibition of bud formation. On the other hand, absence of buds from cultures at higher concentration of glucose (4 per cent) might well be a result of high external osmotic value, since cultures in media in which a similar osmotic concentration was produced by the addition of mannitol also showed complete inhibition of bud formation. The morphological abnormalities encountered in cultures from media containing 4 per cent glucose, are probably also in large part a consequence of the osmotic value of the medium as substantiated by the similarity in protonemal filaments from 4 per cent mannitol, 1 per cent glucose + 3 per cent mannitol, and 4 per cent glucose media (Figs. 13, 15).







FIGS. 10-15.

Discussion

In conformity with the findings of several previous investigators, glucose and sucrose do promote the growth of the protonema and leafy shoot of *Pohlia nutans*. Under certain conditions, addition of sugar to the medium can also advance the date of bud formation and increase the number of buds appearing. These effects of sugars on both protonemal growth and bud formation were most marked under the evidently sub-optimal lighting conditions of cultures in liquid media. The addition of sugars to the medium had comparatively little effect on agar cultures in Petri dishes, which would undoubtedly be exposed to stronger lighting than the liquid cultures in tubes. It, therefore, seems likely that in relatively dim light, bud formation on the protonema may be restricted by failure of the products of photosynthesis, and in particular of the sugars, to attain a certain minimal concentration. This necessary concentration would probably be attained with greater difficulty in liquid media of low osmotic concentration because of the dilution of cell contents by the ready entry of water from the external medium. The relative inhibition of bud formation in dilute liquid media has been noted previously by other authors (Becquerel, 1906; Servettaz, 1913; Pringsheim & Pringsheim, 1935; Meyer, 1940; Kofler, 1956).

Although under sub-optimal lighting conditions, protonemal growth and the formation of shoot-buds was increased by addition of suitable concentrations of sugar (0·5, 1·0 and 2·0 per cent glucose or sucrose), the higher concentration of 4 per cent glucose or sucrose resulted in a great inhibition or complete suppression of bud formation. Pringsheim & Pringsheim (1935) had previously noted that a rich supply of sugar and protein degradation products, e.g. 4 per cent sucrose + yeast autolysate, can entirely suppress the formation of leafy shoots. There is still some doubt as to whether the suppression of buds in concentrated media is an osmotic effect or due to an increase in the internal sugar concentration. In the present investigation, the addition of mannitol to the medium demonstrated that moderate increase in the external osmotic concentration, even in the absence of an external sugar supply, can result in a suppression of bud formation. Whether the effects of a high external osmotic concentration are due to a corresponding increase in the internal osmotic value, or to the associated increase in the concentration of a particular cell solute, cannot be decided from the present data. Changes in the internal osmotic value would certainly affect the hydration level of the protoplasmic and cell-wall colloids. On the other hand, changes in the internal sugar concentration, apart from any osmotic effect, might be expected to affect a considerable range of physiological processes. In a recent investigation of the effects of sugar concentration on the development of two liverworts, *Fossombronia pusilla* and *Reboulia hemisphaerica*, one of us (Allsopp, 1957) observed that the presence of 2 per cent glucose in culture medium destroys the normal polarity of these plants, resulting in the production of masses of undifferentiated callus. It seems likely that the effects of concentrated media in reducing or suppressing the formation of buds on moss protonemata might also result from the disturbance of a polarized flow of nutrients, which is presumably necessary for the initiation of buds of leafy shoots.

The increase in the size of the leafy plants, observed at the lower glucose concentrations in liquid cultures, was

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FIGS. 10-15 — *Pohlia nutans*. Effects of various media on the structure of the protonema. Figs. 10-12. Liquid cultures in daylight; from 0·0, 1·0 and 4·0 per cent glucose media respectively. × 60. Figs. 13-15. Liquid cultures in artificial white light. Nutation patterns in filaments from 4·0 per cent mannitol, 1·0 per cent glucose + 3·0 per cent mannitol and 4·0 per cent glucose respectively. × 110.

probably a consequence of improved carbohydrate nutrition, while the progressive shortening of the axis of leafy plants, together with a reduction in internodal length and leaf size, with further increase in glucose concentration (2·0 and 4·0 per cent), can probably be referred to a supra-optimal carbohydrate supply, and parallels the results obtained previously with the water fern *Marsilea* (Allsopp, 1955). It is also probable that the stunting effect of supra-optimal sugar concentrations follows from a restriction on cellular extension, in part, from the effects of the internal sugar concentration on the synthesis of cell-wall materials and on the hydration of the colloidal constituents of the cell, and partly from the osmotic effects of the concentrated culture medium (Allsopp, 1959). In this connection it should be mentioned that Kofler (1956) discovered that the differentiation of branches of the protonema of *Funaria* was poorly defined in liquid cultures, while Meyer (1940) observed that the leafy shoots of *Physcomitrium turbinatum*, produced in submerged cultures, showed several modifications, with a reduction in the size of the leaves, in the marginal serrations of the leaves and in cellular differentiation. The results obtained by Meyer (1940) under aseptic culture conditions were similar to those obtained previously by Davy de Virville (1927), who studied the effects of reduced light intensity, high atmospheric humidity or aquatic growth on the leafy plants of many moss species. From the present investigation on the effects of sugar concentration on both the protonema and the leafy shoot, it would seem that the instances of reduced differentiation of either protonema or leafy shoot, observed by the cited authors, can all be referred to the production of a suboptimal internal sugar concentration under the particular experimental conditions employed.

In the above account some importance has been attached to the role of sugar concentration in the development of the protonema and in the initiation of buds of leafy shoots. Other evidence indicates, however, that an external carbohydrate supply is unable to substitute for some more specific action of light on the normal development of the protonema and, in particular, on the initiation of buds of leafy shoots. It is true that the formation of buds has been said to occur in complete darkness on protonemata of species of *Splachnum* growing on a 2 per cent sucrose medium (Keil, 1949), but this report appeared only as a short note and further confirmation is desirable. Moreover it may be that the light requirement is different in different ecological groups of mosses. In our own experiment (Mitra *et al.*, 1959), the growth of the protonema on culture media in darkness, although appreciable, was very poor when compared with the amount of growth in light. It is possible that more suitable media might support better growth and even bud formation in darkness, but it seems more likely that normal development of the protonema is dependent upon the action of light, perhaps involving the formation of relatively specific growth regulating substances. This view is supported by observations on the normal development of the protonema. Several workers (e.g. Servettaz, 1913; Sironval, 1947; Bopp, 1957) have commented on the fact that buds are formed only when the protonema has attained a certain level of development, suggesting that the protonema mat must first accumulate a sufficient amount of bud-forming materials. A corresponding delay on all nutrient media hitherto tested suggests that the necessary bud-forming materials are not merely ordinary nutrients but include more specific materials, which can evidently only be synthesized by the protonema in the presence of light. In view of these findings and the other lines of evidence discussed above, a study of the effects of various known physiologically-active substances on the development of the protonema seemed desirable, and are presented in the next paper of this series the results of such an investigation

Summary

The effects of various concentrations of sucrose and glucose on the development of the protonema and bud formation in *Pohlia nutans* were investigated in aseptic cultures. In a parallel series of

experiments, the osmotic concentration of the glucose medium was increased by the addition of mannitol, which is nutritionally inert.

In all concentrations of glucose, sucrose, mannitol, and mannitol + glucose media, the structure of the protonema remained basically the same. In liquid media, there was less differentiation between protonemal filaments but a distinction was still evident between lateral filaments of restricted growth and long primary filaments. Moderate concentrations of the sugars (0·5, 1·0 and 2·0 per cent) led to an increase in the number of buds formed in the early stages of protonemal growth, especially in liquid cultures, but budding was inhibited by higher concentrations of sugars or by corresponding concentrations of mannitol in the media.

Addition of sugar to the medium also promoted the growth of the leafy shoots at low concentrations, but with increasing concentration of the sugar there was a progressive shortening of the axis of the leafy plants, with reduction in internode length and in the dimensions of the leaves.

From a discussion of the present results and earlier observations, it is concluded that although the attainment of a certain minimal sugar concentration in the protonema may be prerequisite for bud formation, it is probable that there is also a requirement for a more specific formative substance which can only be synthesized by the protonema in the presence of light.

G. C. Mitra is greatly indebted to Professor C. W. Wardlaw for providing facilities in the laboratory and for his encouragement throughout the investigation. The kind assistance of Mr E. Ashby and Mr G. Barker in preparing the photographic illustrations is acknowledged with many thanks.

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III. THE EFFECTS OF VARIOUS PHYSIOLOGICALLY ACTIVE SUBSTANCES ON THE DEVELOPMENT OF THE PROTONEMA AND BUD FORMATION IN *POHLIA NUTANS* (HEDW.) LINDB.

G. C. MITRA* & A. ALLSOPP

Department of Cryptogamic Botany, Manchester University, Manchester, U.K.

Previous studies on the effects of various qualities of light (Mitra *et al.*, 1959) and of sugar concentrations (Mitra & Allsopp, 1959) on the development of the protonema and on the formation of buds in *Pohlia nutans* have shown that both these processes are greatly influenced by exposure to light. These experiments also indicate that the action of light is not merely a trophic one, but a more specific stimulus, possibly dependent on the production of one or more formative substances. It, therefore, seemed of interest to study the effects of various physiologically active substances on *Pohlia nutans*, particularly since there have been relatively few studies on the effects of growth substances on the development of the Bryales. Relevant earlier works are considered later in the discussion of our own results.

Material and Methods

The basic medium, material and procedure were similar to those described earlier (Mitra *et al.*, 1959). Agar cultures in Petri dishes, tubes and flasks, and liquid cultures in tubes were grown in artificial light of relatively high intensity (375 foot candles) and at a temperature of 22°-24°C.

The following concentrations of growth substances were employed: indoleacetic acid (IAA) at 10, 1·0 and 0·1 mg/l; indoleacetonitrile (IAN) at 50, 10, 1·0 and 0·1 mg/l; α-naphthaleneacetic acid (NAA) at 10, 1·0 and 0·1 mg/l; kinetin at 10, 1·0 and 0·1 mg/l; kinetin at the same concentrations with 0·1 mg/l IAA; and gibberellic acid (GA) at 100, 10, 1·0 and 0·1 mg/l.

Experimental Results

Effects of Indoleacetic Acid (IAA), Indoleacetonitrile (IAN), α-Naphthaleneacetic Acid (NAA) — These three growth substances are treated together, since a comparison was made between the effects of these substances in three parallel series of cultures. Some of the results are presented in Table 1 and illustrated by Fig. 1.

Some protonemal growth was obtained at the concentrations 0·1, 1·0 and 10 mg/l of all three growth substances, but there was no growth in IAN at 50 mg/l. Growth was poor at all concentrations of NAA, especially at 10 mg/l, and was also restricted in both IAN and IAA at 10 mg/l. In all the cultures, except the attenuated ones in NAA at 10 mg/l and 1 mg/l, the protonema had the characteristic heterotrichous habit, with differentiation into erect and prostrate systems of filaments. The relative development of the two systems differed according to the nature and concentration of the growth substance. In IAA and IAN at concentrations of 10 mg/l and 1 mg/l the erect system was more feebly developed than in the control cultures. In NAA at 0·1 mg/l a few elongated erect filaments appeared. At all

*Present Address: National Botanic Gardens, Lucknow, India. This investigation was carried out during the tenure of a Fellowship under the Colombo Plan.

TABLE 1 — EFFECTS OF DIFFERENT CONCENTRATIONS OF IAN, IAA AND NAA ON BUD FORMATION IN *POHLIA NUTANS*

	Number of buds (Mean value of 3 cultures, value from liquid cultures in brackets)			
Dates*	14-10-56	22-10-56	1-11-56	14-11-56
Control	6·2(0)	14·5(6·2)	24·3(11·6)	41·6(16·3)
IAN				
50 mg/l	0(0)	0(0)	0(0)	0(0)
10 ,,	0(0)	2·4(0)	4·7(0)	7·3(0)
1 ,,	6·9(0)	12·5(3·0)	21·3(7·8)	43·1(13·2)
0·1 ,,	7·0(0)	13·7(5·1)	22·8(9·3)	40·6(15·6)
IAA				
10 mg/l	0(0)	0(0)	0(0)	0(0)
1 ,,	0(0)	2·5(0)	6·6(0)	10·4(0)
0·1 ,,	0(0)	10·6(0)	19·3(4·7)	39·5(10·2)
NAA				
10 mg/l	0(0)	0(0)	0(0)	0(0)
1 ,,	0(0)	0(0)	0(0)	0(0)
0·1 ,,	0(0)	0(0)	0(0)	0(0)

*Cultures started on 29-9-56.

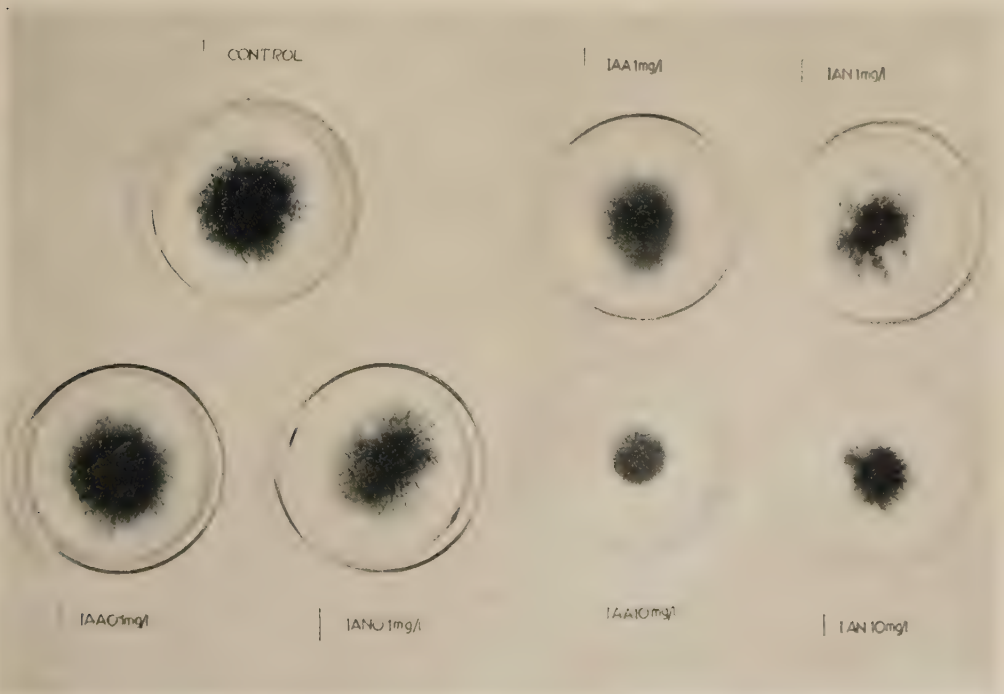

FIG. 1 — *Pohlia nutans*. Effects of various concentrations of IAA and IAN on protonema growth and bud formation. Cultures grown under artificial white light. × $\frac{2}{3}$ natural size.

concentrations of NAA, the protonemal filaments were pale yellowish-brown, partly on account of the wall pigmentation, and also because the chloroplasts were reduced to pale yellow-green structures.

The formation of shoot-buds on the protonema was greatly influenced by the presence of the growth substances. It is evident from the data shown in Table 1 that in no case did the presence of any of the three growth substances have any stimulatory action on bud formation. On the contrary, at certain concentrations, there was a marked inhibitory effect. Normal buds were never formed at any concentration of NAA, although microscopical examination of cultures in the lowest concentration of NAA (0·1 mg/l) revealed a few arrested buds. Bud suppression was also complete in IAA at 10 mg/l, while in IAN at the same concentration the number of buds was considerably reduced and the time of their first appearance delayed by a week as compared with the control cultures. At 1 mg/l and 0·1 mg/l, IAN had little effect on bud formation, while IAA at 1 mg/l had a distinct inhibitory effect. At 0·1 mg/l, IAA led to some delay in the appearance of buds, but the final number produced was virtually the same as in the control cultures. With both IAA and IAN, as well as in the control cultures, buds always appeared earlier on agar cultures than in liquid media. In liquid media, even 0·1 mg/l IAA had a marked inhibitory action on bud formation.

Effects of Kinetin, Kinetin + Indoleacetic Acid and Gibberellic Acid — Many recent investigations have demonstrated the striking effects of kinetin and gibberellic acid on various growth processes of flowering plants, but at the beginning of the present investigation only a brief note had been published on the action of 6-(substituted)-aminopurines, including kinetin, on bud development in mosses (Skiner & Shieve, 1955), and apparently there had been no report of the effects of gibberellic acid on mosses. In the present work, it has been found that kinetin and gibberellic acid have marked effects on protonemal growth and on the development of buds of leafy shoots in *Pohlia nutans*.

For the first two weeks after inoculation, there was little difference between the various cultures, but with further growth, differences became increasingly evident. Some of the effects of GA, kinetin, kinetin + IAA and IAA alone are summarized in Table 2 and illustrated by Figs. 2 and 3. It is shown that maximum horizontal spread of the protonema occurred in control cultures and at lower concentrations of GA (10 mg/l, 1 mg/l and 0·1 mg/l). Bud formation was affected by both GA and kinetin and by kinetin + IAA. It will be seen from Table 2 that the presence of kinetin led to the production of a large number of bud initials after about 17 days of growth. Buds appeared after a further period of 10 days in cultures containing 100 mg/l GA, but approximately a further fortnight elapsed before buds were observed in the remaining cultures, i.e. in lower concentrations of GA, IAA only and in the control cultures. The number of buds produced in the cultures supplied with kinetin, kinetin + IAA or GA (100 mg/l) was so large that it was impracticable to make accurate counts. It is also of interest that buds appeared in the cultures containing kinetin or GA (100 mg/l) when the diameter of the protonemal mat was only about one-third that of the control-protonemal mat at the time of bud formation.

Morphological examination of the protonema revealed that neither GA nor kinetin had produced any visible abnormalities in the protonemal filaments, but although kinetin stimulated the production of shoot-buds, it had evidently an

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Fig. 2 — *Pohlia nutans*. Effects of various concentrations of kinetin, kinetin + IAA and IAA on protonema growth and bud formation. Cultures grown under artificial white light. *a*, control; *b*, 0·1 mg/l IAA; *c*, 0·1 mg/l kinetin + 0·1 mg/l IAA; *d*, 0·1 mg/l kinetin; *e*, 10 mg/l kinetin + 0·1 mg/l IAA, and *f*, 10 mg/l kinetin. × $\frac{3}{7}$ natural size.

TABLE 2 — EFFECTS OF VARIOUS GROWTH SUBSTANCES ON THE RADIAL GROWTH OF THE PROTONEMA AND BUD FORMATION

Mean diameter of cultures in mm and No. of buds (B)† in brackets*

Dates‡	31-8-57	10-9-57	25-9-57	11-10-57
Gibberellic acid				
100 mg/l	15	27 (large No. B)	38	54
10 ,,	19	35	53(15·3B)	67
1 ,,	22	35	50(17·8B)	64
0·1 ,,	22	33	50(12·2B)	64
Kinetin				
10 mg/l	14 (large No. B)	22	35	50
1 ,,	15 do	24	37	47
0·1 ,,	16 do	24	37	51
Kinetin + 0·1 mg/l *IAA*				
10 mg/l + 0·1 ,,	18 (large No. B)	29	41	55
1 ,, + 0·1 ,,	17 do	25	36	54
0·1 ,, + 0·1 ,,	17 do	27	42	56
IAA				
0·1 mg/l	17	28	40(7·8B)	56
Control	19	32	49(9·6B)	67

*Mean measurements of three similar petri dish cultures.
†(B) indicates the appearance of buds for the first time in each culture.
‡ Culture started on 14-8-57.

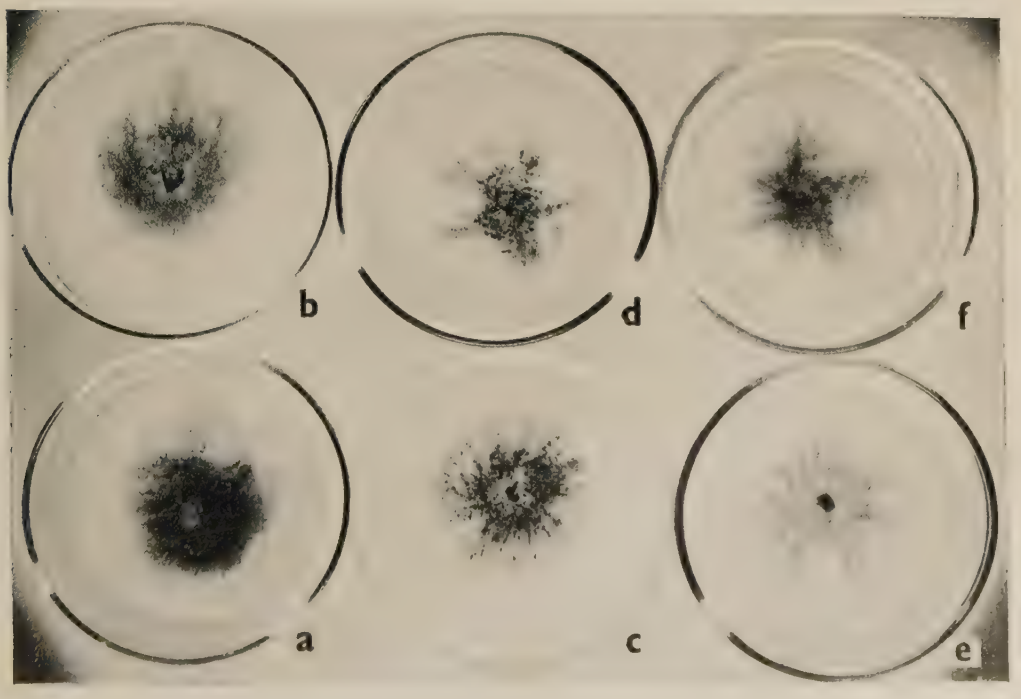

FIG. 2.

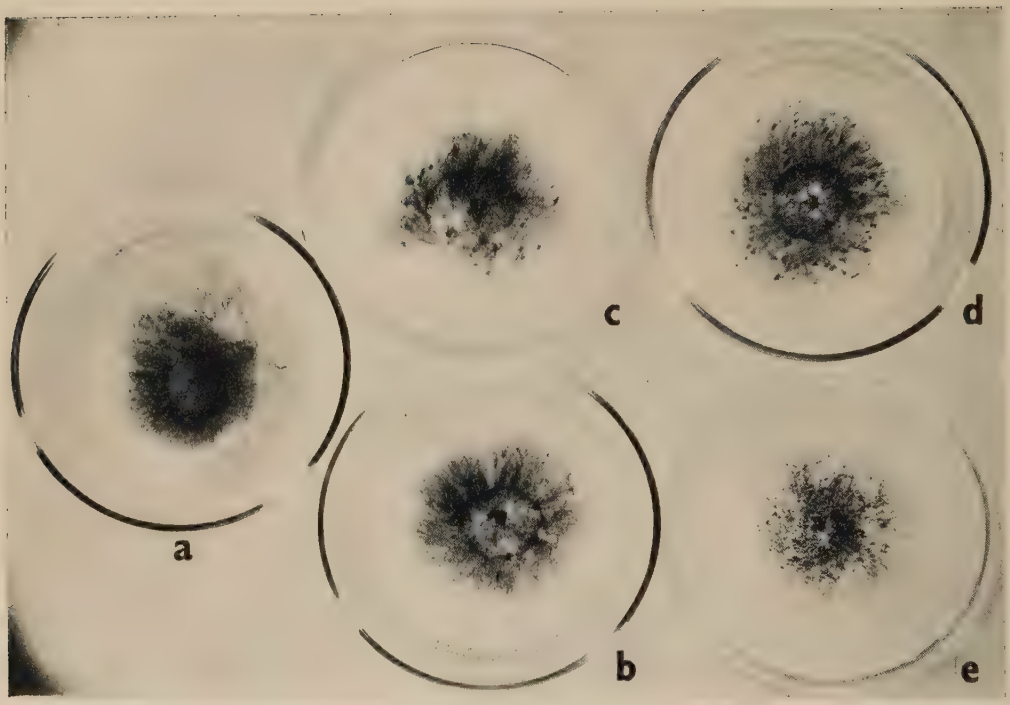

FIG. 3 — *Pohlia nutans*. Effects of various concentrations of gibberellic acid on protonema growth and bud formation. Cultures grown under artificial white light. *a*, control; *b*, 0·1 mg/l; *c*, 1 mg/l; *d*, 10 mg/l and *e*, 100 mg/l. × $\frac{3}{7}$ natural size.

adverse effect on their subsequent development. The majority of buds from media containing kinetin (10 mg/l and 1 mg/l), with and without IAA, ultimately became brown and remained stunted; sometimes producing a callus-like mass of cells. The production of several stunted shoots from a common basal mass of cells was also frequent. With a lowering of the concentration of kinetin, there was a decrease in the proportion of abnormal shoots with a progressive increase in the number of normal leafy plants, and at 0·1 mg/l kinetin, normal plants were predominant. At the latter concentration of kinetin, addition of 0·1 mg/l IAA increased the number of normal leafy plants, but this concentration of IAA could not counteract the adverse effects on bud development of the two higher concentrations of kinetin.

Gibberellic acid, even at 100 mg/l, had little adverse effect on bud development. At no concentration of GA did the leafy shoots display any tendency towards an increase in internode length; in fact at 100 mg/l GA the leafy shoots were somewhat dwarfed as compared with control cultures.

Discussion

The results of the present investigation have shown that in *Pohlia nutans*, although the protonema itself is relatively little influenced by the presence of various growth substances, bud formation is greatly affected. Of the growth substances supplied, those of the auxin-type, viz. IAA, IAN and NAA, were inhibitory to bud formation at relatively low concentrations, while kinetin and gibberellic acid, at appropriate concentrations, had a pronounced stimulatory action on the initiation of buds of leafy shoots.

Most of the previous investigations on the effects of substances of the auxin type on the Bryales have been mainly concerned

with effects on spore germination or on the polarity and early growth of the protonema (Heitz, 1942; Fries, 1943; Bünning & Wettstein, 1953; Wettstein, 1953; Benson-Evans, 1953; Patterson, 1957), but some authors have studied the effects of auxin on bud formation. Thus, Hurel-Py (1948, 1953) found that although the protonema of *Funaria* and other mosses can continue growth on media containing 10 mg/l NAA, buds of leafy shoots are not produced at that concentration, but do appear when the concentration of NAA is reduced to 0·1 or 0·001 mg/l. Bopp (1953) studied the effects of IAA on bud formation on the primary and secondary protonema of *Funaria*. He found that at 0·01 mg/l IAA the number of buds was slightly increased as compared with the control cultures, but that bud formation was inhibitory at concentrations of 1 and 10 mg/l IAA. Confirmation of the effect of moderate concentrations of IAA and NAA in suppressing the formation of shoot-buds on the protonema was provided by Gorton & Eakin (1957) in investigations on *Tortella caespitosa*. The results of the present work on the effects of IAA and NAA on *Pohlia nutans* are thus in agreement with the findings of other investigators.

There has been no previous study, however, of the effects of the naturally occurring growth substance IAN on representatives of the Bryales. Like the other substances of the auxin-type, IAN inhibited the development of shoot-buds on the protonema, but at corresponding concentrations its action in this respect was only about one-tenth as effective as IAA, a result in agreement with the relative effects of IAA and IAN on the development of lateral buds in the water fern *Marsilea* (Allsopp, 1956).

The results obtained in the present work, and in the studies of previous authors, thus provide no indication that substances of the auxin-type play any important role in the initiation of shoot-buds on the protonema of the Bryales, but our experiments with kinetin and with gibberellic acid suggest that similar growth factors may be of considerable significance in the normal progress of bud initiation. The effects of kinetin on the development of the protonema and the formation of shoot-buds have been investigated previously by Gorton & Eakin (1957) and Gorton *et al.* (1957) in their studies on *Tortella caespitosa*. Their results were similar in several respects to those observed in the present investigation. Thus, it was found that while kinetin has a marked effect in stimulating bud formation it has an adverse effect on the subsequent development of the shoot, resulting in the production of various abnormalities. In contrast to our own findings, however, Gorton & Eakin observed that none of the various substances investigated by them, including kinetin, was successful in shortening the time between germination and budding. They also found that after the protonemal mat had attained a maximum diameter of 10-20 mm, further growth of the protonema entirely ceased. On the basis of these results, a scheme for the mechanism of bud development was proposed. It was considered that the condition of " ripeness to bud " is attained only after the protonema has passed through a certain growth period involving (a) the building-up of a store of nutrients to provide for the rapid cell division and synthesis during budding, and (b) the accumulation of growth factors affecting budding, including the excretion of a growth inhibitor into the medium. Gorton & Eakin attached great importance to this inhibitor believing that the origin of the more or less spherical cells from which the buds are produced is dependent on the accumulation of the inhibitor in the medium to a concentration at which cell elongation is arrested. Kinetin was considered to function as the growth factor promoting the active cell division by which the solid tissue mass of the bud is produced from the somewhat spherical initial cell.

Several results of the present investigation are in opposition to the views of Gorton & Eakin on bud formation. In the first place, kinetin definitely advanced the date of bud formation as compared with the onset of this process in the control cultures. Secondly, the production of buds was not accompanied by any immediate cessation of growth in the protonemal mat, i.e. there was no evidence

that the production of buds was connected in any way with the excretion of a growth inhibitor. Furthermore, kinetin greatly increased the number of buds and not merely the rate of development of established initials.

Our results with kinetin are supported by similar results with gibberellic acid (at the higher concentration of 100 mg/l), which also advanced the date of bud formation and led to an increase in the number of bud initials. There have apparently been no previous investigations on the effects of GA on bud formation on moss protonemata, although Maltzahn & MacQuarrie (1958) studied the effect of GA on the growth of the protonema of *Splachnum ampullaceum*. The rate of protonemal growth recorded by these authors was so low, however, even on stimulation by GA, as to suggest that the conditions of growth were distinctly suboptimal.

The stimulation of bud development on the protonema of *Pohlia nutans* by both kinetin and GA is of particular interest, since it has been found by a number of authors (Miller, 1956; Scott & Liverman, 1956; Kuraishi & Hashimoto, 1957; Brian, 1958) that several of the physiological responses of flowering plants to these substances are similar to those induced by red light. In the mosses, it has been known since the early work of Klebs (1893) that light is normally necessary for the development of shoot-buds on the protonema, and in the two previous papers on *Pohlia nutans* (Mitra *et al.*, 1959; Mitra & Allsopp, 1959) it was shown that bud development was dependent on exposure to red light, which could not be replaced by the supply of glucose or sucrose in the external medium. From the result of the present work, it seems likely that, as in flowering plants, the supply of kinetin or gibberellic acid to the protonema may eliminate or reduce the red light requirement, although the action of these substances on dark-grown protonemata has still to be tested. Substances of similar nature are probably involved in the normal process of bud initiation on the protonema, but, as in the flowering plants, the respective roles of kinetin and gibberellic acid are still unknown, particularly since these growth factors are dissimilar chemically and are effective at very different concentrations.

Summary

The effects of different concentrations of various physiologically active substances, viz. α-naphthaleneacetic acid (NAA), indoleacetic acid (IAA), indoleacetonitrile (IAN), kinetin, kinetin + IAA and gibberellic acid (GA) on the development of the protonema and the initiation of shoot-buds in *Pohlia nutans* were studied in aseptic culture.

The morphological pattern of the protonema was little affected by any of these substances apart from NAA, which at the three concentrations tested (10, 1 and 0·1 mg/l) resulted in the development of an attenuated, pale yellow-brown protonema, lacking the normal differentiation into prostrate and erect filaments.

The higher concentrations of the three auxins employed (NAA, IAA and IAN) had a marked inhibiting effect on the formation of shoot-buds on the protonema. With NAA, inhibition was complete at 10 and 1 mg/l but a few arrested buds were observed at 0·1 mg/l. At three concentrations 10, 1 and 0·1 mg/l, IAA had some inhibitory action, although this was little marked at 0·1 mg/l. With IAN there was no inhibition of bud formation at 0·1 and 1 mg/l, but at 10 mg/l the inhibition was comparable to that obtained with IAA at 1 mg/l.

All the concentrations of kinetin employed (10, 1 and 0·1 mg/l), whether with or without the addition of 0·1 mg/l IAA, resulted in the early appearance of a large number of shoot-buds on the protonema. Similar early induction of buds was observed in cultures containing 100 mg/l GA, but at other concentrations (10, 1 and 0·1 mg/l) it had no appreciable effect on budding. Buds appeared in the control cultures only when the diameter of the protonemal mat was approximately three times that of the cultures containing kinetin or 100 mg/l of GA.

The experimental results are discussed in relation to other studies dealing with bud formation in mosses, and it is suggested that kinetin or gibberellic acid may

replace, at least in part, the action of red light, previously found necessary for the development of shoot-buds on the protonema of *Pohlia nutans*, when grown under aseptic conditions on ordinary culture media.

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L'ANATOMIE VASCULAIRE ET L'INTERPRÉTATION DE LA FLEUR PISTILLÉE DE L'*HILLEBRANDIA SANDWICENSIS* OLIV.

ROGER GAUTHIER

Institut Botanique, Université de Montréal, Canada

Le genre *Hillebrandia* appartient à la famille des Bégoniacées. Il compte une seule espèce, *Hillebrandia sandwicensis* Oliv., et cette unique espèce se rencontre seulement dans quelques-unes des îles de l'archipel d'Hawaii, autrefois les îles Sandwich, — d'où le nom spécifique.

L'*Hillebrandia* ressemble beaucoup à un *Begonia* (MacCaughey, 1918; Degener, 1938). Toutefois la fleur pistillée (Figs. 15, 16) diffère, par un certain nombre de caractères, de celle des *Begonia* communément cultivés. Elle est dépourvue d'ailes, ce qui lui donne une apparence plus simple (si l'on exclut les grandes bractées colorées). Le périanthe des *Begonia* comprend seulement un verticille. Chez l'*Hillebrandia*, au contraire, on trouve calice et corolle: cinq pièces rudimentaires, considérées comme des pétales, alternent avec cinq sépales pétaloïdes. Le nombre des pièces de chaque verticille est le même que celui des loges à la base de l'ovaire et des bourrelets placentaires, ce qui paraît plus régulier que de présenter, comme beaucoup de *Begonia*, un ovaire à trois loges et un périanthe de cinq ou de deux pièces. Enfin, chez l'*Hillebrandia*, l'ovaire est incomplètement infère; la placentation, axile[1] à la base sur une courte distance, est pariétale dans la partie supérieure; et les marges d'un même carpelle, bien que rapprochées au sommet, demeurent libres l'une de l'autre, de sorte que l'ovaire n'est pas fermé.

Le présent travail a pour but de décrire la course des faisceaux vasculaires de la fleur de l'*Hillebrandia* et d'en proposer une interprétation. Sur la validité des conclusions obtenues par cette méthode, on trouvera quelques considérations dans Arber (1954). Dans notre opinion, la disposition des faisceaux vasculaires révèle certaines particularités (symétrie, connexions, bifurcations, etc.) qui reçoivent une explication si l'on admet que la fleur correspond à l'extrémité d'un axe portant des appendices, que ces appendices sont homologues (Arber, 1937, 1950) de feuilles, qu'ils présentent des concrescences avec les autres appendices du même verticille et avec ceux des autres verticilles localisés dans le même secteur.

Matériel et Technique

En 1946, l'auteur obtenait des spécimens de l'*Hillebrandia sandwicensis* par l'entremise du Dr. Harold Saint John, directeur du Department de Botanique de l'Université d'Hawaii. Ces spécimens provenaient de l'île Maui et portaient les indications suivantes: Koolau Gap, Mount Haleakala, 5700 pieds d'altitude, 2 septembre 1945. H. St. John et A. L. Mitchell, No. 21, 271. Une dizaine de fleurs ont été étudiées par la méthode des coupes en série.

Plus récemment, l'*Hillebrandia* a été introduit au Jardin Botanique de Montréal par M. Henry Teuscher, conservateur du Jardin. Au printemps 1958, les plantes ont abondamment fleuri, ce qui a permis d'observer du matériel frais et d'obtenir de nouvelles coupes. Les photographies et la plupart des photomicrographies qui accompagnent cet article proviennent de cette source.

Entre les fleurs reçues directement d'Hawaii et celles provenant du Jardin Botanique, il n'y a dans la structure

1. Le terme "axile" est pris au sens purement descriptif qu'on lui donne habituellement (Lawrence, 1951).

vasculaire aucune différence fondamentale. Dans les deux cas, mais surtout dans les plantes en culture, un certain nombre de fleurs n'avaient que quatre carpelles. On peut y voir l'expression d'une tendance qui se retrouve aussi dans le genre *Begonia*, où la plupart des espèces ont seulement trois carpelles. D'autres variations occasionnelles se présentent, mais elles n'affectent pas le schéma général du squelette vasculaire de la fleur.

A mesure que les graines se développent et que le fruit parvient à maturité, les parenchymes qui entourent les faisceaux de la fleur se lignifient, surtout à la base de l'ovaire. Les fleurs étudiées étaient en général bien épanouies, mais n'avaient pas atteint ce stade. Les coupes, épaisses de 12 à 25 μ, ont été colorées soit à la safranine-vert acide ("Fast green F C F"), soit au violet cristal-érythrosine.

Saunders (1931) a déjà donné une brève description de l'anatomie florale de l'*Hillebrandia*. En conformité avec sa théorie du polymorphisme carpellaire, elle interprète la fleur comme formée de deux verticilles de carpelles, les uns stériles, les autres fertiles. Il n'y a pas lieu de revenir ici sur cette description, non plus que de reprendre la discussion de la théorie sur laquelle elle est basée. Quant au problème connexe des *Begonia*, on le trouvera discuté dans Bugnon (1926), Hall (1949), Gauthier (1950), Puri (1952a, 1952b), Bugnon et Bugnon (1953), Douglas (1944, 1957), Leinfellner (1954).

Observations et Interprétation

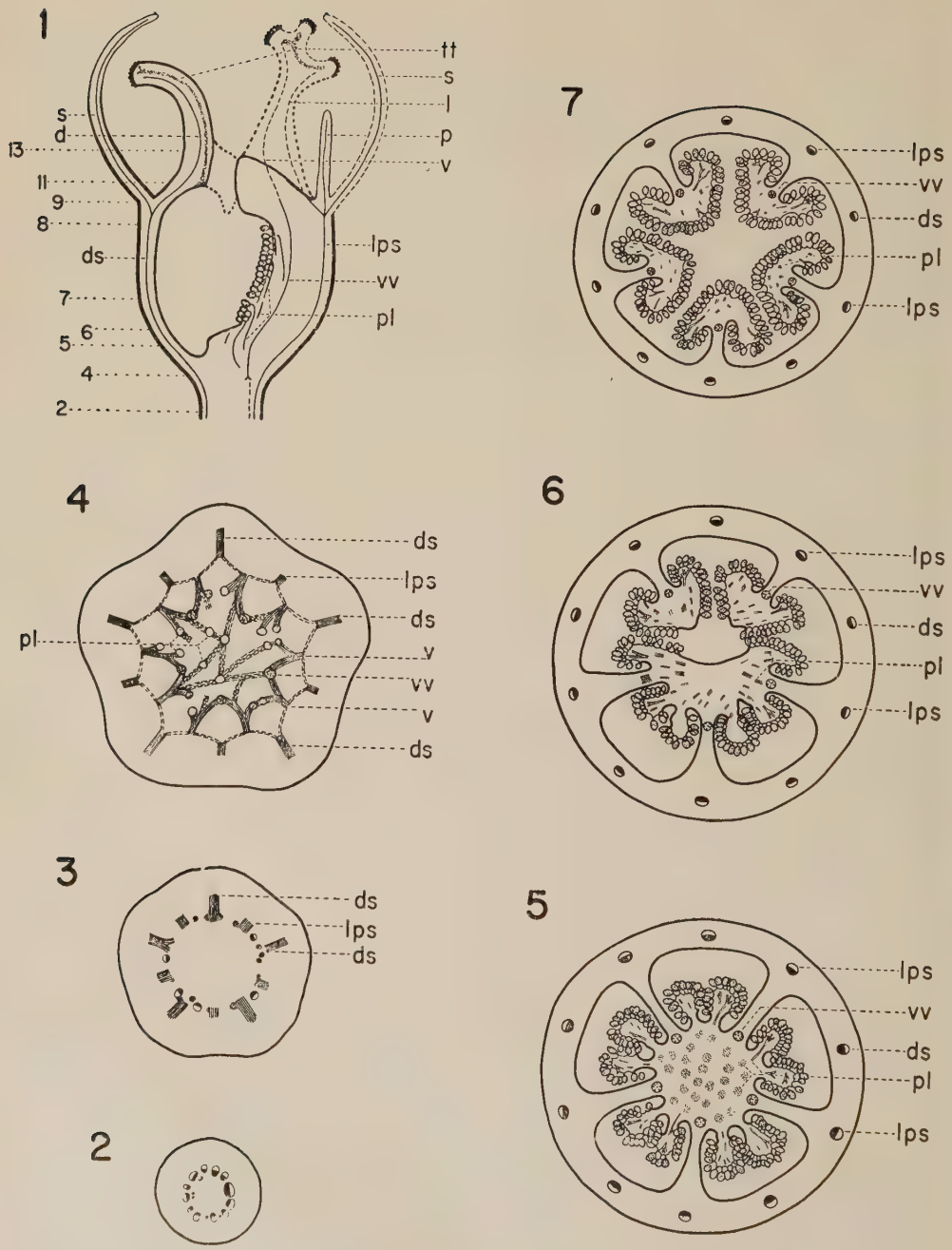
La base de la fleur — En coupe transversale (Figs. 2, 17), le pédicelle présente une série de faisceaux vasculaires distincts. C'est la structure que les auteurs appellent dictyostèle (Eames & MacDaniels, 1947), ou eustèle (Esau, 1953).

Au niveau du réceptacle, cinq faisceaux vasculaires quittent la stèle (Fig. 3, *ds*), passent[2] dans le parenchyme périphérique et montent dans la paroi externe de l'ovaire (Fig. 1). On peut les voir dans les coupes suivantes, d'abord vis-à-vis le milieu de chacune des loges (Fig. 5), puis à mi-chemin entre les bourrelets placentaires (Fig. 7). Chacun de ces cinq cordons vasculaires représente le faisceau dorsal d'un carpelle ainsi qu'un faisceau destiné au sépale opposé. Bien qu'elle soit figurée dans un même schéma, l'insertion des faisceaux *ds* ne se fait pas tout à fait au même niveau: elle suit une spirale très aplatie (Fig. 18), comme il arrive souvent dans les organes floraux (Eames & MacDaniels, 1947).

Cinq autres cordons vasculaires (Fig. 3, *lps*) prennent ensuite naissance, à mi-distance entre les dorsaux. Leur course ultérieure amène à conclure que chacun représente deux faisceaux latéraux de carpelles contigus, le faisceau d'un pétale et deux faisceaux appartenant à des sépales voisins. Tout comme les dorsaux, les cordons vasculaires *lps* passent dans le parenchyme périphérique. On les retrouve à la limite théorique des carpelles, sur un même cercle que les dorsaux *ds*, mais vis-à-vis les cloisons interloculaires (Figs. 4, 5, etc.). Les faisceaux *ds* et *lps* peuvent se détacher du côté ou du milieu d'un faisceau de la stèle, ou être constitués par un faisceau qui passe tout entier dans la fleur.

Pendant que s'organisent ces cordons externes, des modifications profondes surviennent dans la région centrale de la stèle (Figs. 4, 19): elles aboutissent à la formation des faisceaux *vv*, représentant les ventraux de deux carpelles adjacents, et des faisceaux placentaires (*pl*). Ces faisceaux deviennent bientôt distincts (Fig. 5), mais leur origine est difficile à mettre en évidence. La figure 4 illustre ce qui paraît être le schéma général. De part et d'autre de chaque dorsal, un faisceau se dirige vers la région médullaire de la stèle. Ainsi prennent naissance, dans chacun des carpelles, deux cordons vasculaires, les faisceaux ventraux (*v*). Dès son origine, le ventral émet des ramifications vers le carpelle auquel il appartient: il s'agit des faisceaux placentaires (*pl*), qui viennent se placer sur un même rayon que le dorsal, entre celui-ci et le centre de l'ovaire, et d'où se détacheront

2. Ce terme, ainsi que les autres qui décrivent la course des faisceaux vasculaires, est purement descriptif: il n'implique aucune idée de mouvement ni de polarité dans la différenciation des faisceaux.



Figs. 1-7 - Coupes schématiques de la fleur pistillée de l'*Hillebrandia sandwicensis*. Fig. 1. Coupe longitudinale, dans un plan passant par un faisceau *ds* et un faisceau *lps*. Les chiffres à gauche de cette figure indiquent le niveau approximatif des coupes transversales correspondantes. Les parties en pointillé ne sont pas tout à fait dans le plan de la coupe. Figs. 2-7. Coupes transversales, à des niveaux successifs à partir de la base. Dans les faisceaux *ds* et *lps*, le xylème est en noir et le phloème en blanc. Les faisceaux ventraux et les faisceaux placentaires sont en pointillé. Pour détails, voir le texte.

les traces ovulaires. Chaque ventral s'écarte du dorsal et se dirige obliquement vers le carpelle voisin. Il s'unit bientôt au ventral de ce carpelle et forme avec lui un cordon vasculaire unique (*vv*), localisé à la limite théorique des deux carpelles, juste à la base de la cloison interloculaire. D'autres rameaux placentaires partent des faisceaux ventraux, de sorte que le centre de l'ovaire présente bientôt l'aspect des figures 5 et 20.

En plus des cordons vasculaires principaux, qui donnent naissance aux différents faisceaux de la fleur, on en distingue assez souvent quelques autres, généralement plus petits (Figs. 2, 3). Ils disparaissent dans les nombreuses anastomoses qui relient les faisceaux (Fig. 4) et qui marquent le sommet de l'axe. Au-dessus de ce niveau, on ne trouve que les faisceaux des carpelles et du périanthe.

La région à placentation axile — Tous les faisceaux de la fleur ont maintenant pris naissance. Une coupe transversale de l'ovaire offre alors l'aspect de la figure 5, qu'il y a lieu de considérer plus en détail. Cinq loges (en réalité, cinq lobes d'une cavité ovarienne unique) sont maintenant visibles dans l'ovaire. Chacune présente dans son angle interne deux marges placentaires portant un grand nombre d'ovules. Dans la paroi externe montent dix cordons longitudinaux: cinq, vis-à-vis le centre de chaque loge (les faisceaux *ds*); cinq, vis-à-vis chaque cloison interloculaire (les faisceaux *lps*). Dans chacune des cloisons, sur un même rayon que les faisceaux *lps*, mais plus au centre, montent cinq autres cordons longitudinaux (les faisceaux *vv*). A ce niveau la placentation est axile: les ovules paraissent attachés sur une colonne située au centre de l'ovaire et parcourue dans toute sa masse par les nombreux faisceaux placentaires *pl*, issus directement ou indirectement des faisceaux ventraux (Figs. 5, 20).

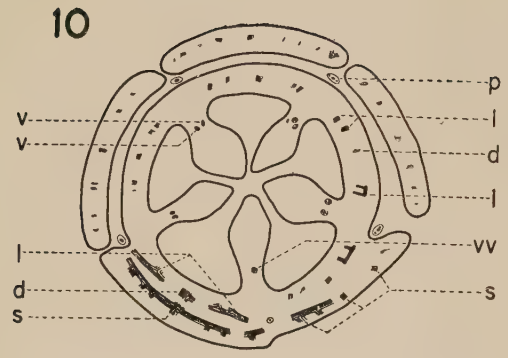
Abstraction faite du périanthe, pareille structure peut s'interpréter comme un ovaire formé de cinq carpelles unis latéralement. Chaque carpelle est homologue d'une feuille et possède les faisceaux suivants: un faisceau dorsal; deux faisceaux latéraux, unis congénitalement aux faisceaux latéraux des carpelles voisins; deux faisceaux ventraux, fusionnés avec les ventraux des carpelles adjacents. Le milieu du carpelle est marqué par le dorsal, représenté par le faisceau *ds*.

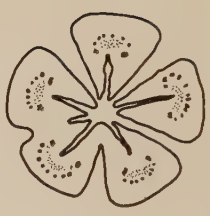
Dans les faisceaux ventraux (*vv*), la position relative du xylème et du phloème est difficile à déterminer. Elle paraît plus ou moins constante. L'auteur n'a pas jusqu'ici observé l'inversion caractéristique de ces faisceaux.

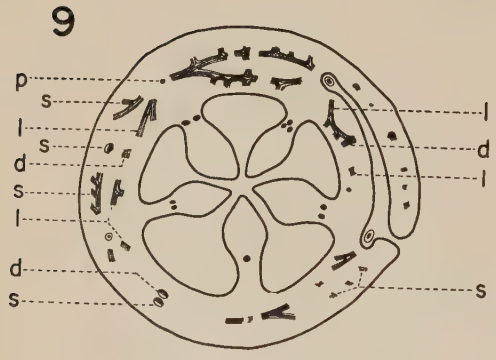
Le passage à la placentation pariétale — La colonne placentaire centrale fait maintenant place à cinq secteurs indépendants (Figs. 6, 7, 21, 22). La placentation, axile depuis la base, devient pariétale, et les ovules forment cinq bandes longitudinales le long de la paroi de l'ovaire (Fig. 23). Chacun des secteurs placentaires qui se dégagent à ce niveau offre en coupe transversale l'aspect d'un fer de lance. Il est constitué d'un bourrelet charnu localisé à la marge de deux carpelles adjacents. Un faisceau *vv* monte dans la cloison vis-à-vis chacun des placentas.

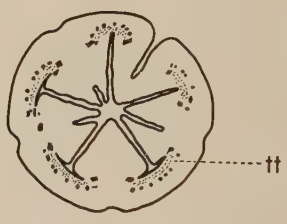
Si l'on admet la théorie carpellaire, ce passage d'un type de placentation à l'autre est facile à comprendre: il s'explique par la concrescence plus ou moins poussée des marges carpellaires (ce terme incluant ici les placentas charnus). Dans la partie où la placentation est axile, l'union existe non seulement entre les marges des carpelles voisins, mais aussi entre celles d'un même carpelle. Chaque carpelle est une structure close, et l'ovaire à ce niveau est pluriloculaire. Dans la partie où la placentation est pariétale, la concrescence va moins loin. Les carpelles adjacents demeurent unis, mais les bords d'un même carpelle sont libres l'un de l'autre. L'ovaire est alors uniloculaire, et le pistil paraît formé d'unités soudées latéralement, mais non refermées sur elles-mêmes.

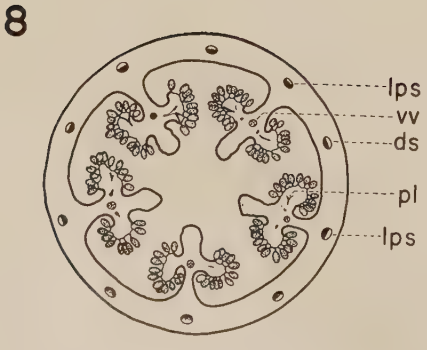
Malgré les apparences, la colonne centrale n'est pas un axe. Elle équivaut aux cinq bourrelets indépendants auxquels elle fait place, elle représente la base de tous les placentas. La course des faisceaux vasculaires l'avait déjà indiqué. Le passage que l'on observe entre les deux types de placentation le corrobore (Figs. 21, 22): la transition est si graduelle que le trajet des faisceaux placentaires est à peine modifié. Toute hypothèse qui ne

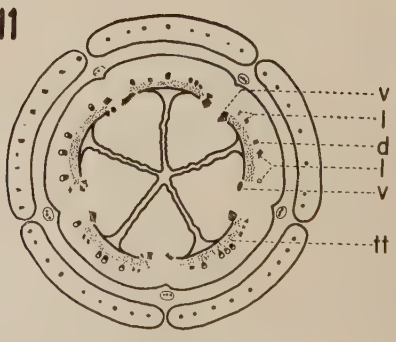












FIGS. 8-14 — Coupes transversales schématiques de la fleur pistillée de l'*Hillebrandia sandwicensis*. Dans les figures 9 et 10, les ovules ne sont pas représentés. Pour détails, voir le texte.

considère pas le centre du pistil comme étant de nature appendiculaire rend difficilement compte de cette disposition des placentas: on ne voit guère comment une colonne placentaire de nature axiale se prolongerait en cinq placentas indépendants le long de la paroi ovarienne.

La présence des deux types de placentation — axile et pariétale — est à rapprocher de certaines particularités de la fleur des *Begonia*. Chez les *Begonia*, la notion de carpelles homologues de feuilles permet de comprendre l'existence de placentas simples et bifides. Elle explique aussi la communication entre les loges dans la partie supérieure de l'ovaire. Retenons ce dernier caractère. Au niveau où les loges communiquent entre elles, les carpelles des *Begonia* sont unis latéralement, mais les deux marges de chaque carpelle ne sont pas soudées (Gauthier, 1950, pp. 61-63, et Fig. 40). Il suffirait d'agrandir vers le bas et de prolonger vers le haut cette ouverture, et l'on obtiendrait la condition qui prévaut dans l'*Hillebrandia*. L'ovaire des *Begonia* (exception faite de la section *Mezierea*) est pluriloculaire et à placentation axile, celui de l'*Hillebrandia* est uniloculaire et à placentation pariétale, sauf à la base. Ils deviennent cependant comparables, si l'on admet l'interprétation proposée. La différence entre les deux provient de l'union plus ou moins complète des marges des carpelles. La même notion rend compte du fait que l'ovaire de l'*Hillebrandia* est ouvert au sommet.

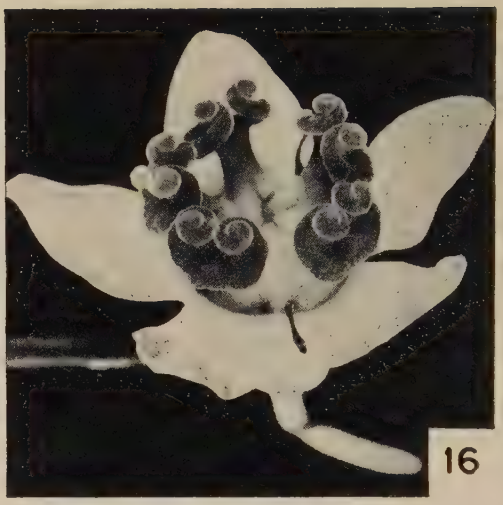
Le périanthe — La structure illustrée dans les figures 7 et 23, et décrite précédemment, se maintient jusqu'au point où sépales et pétales se détachent du pistil (Figs. 1, 8, 9, 10). Il y a cinq sépales, opposés aux carpelles. Cinq autres pièces, beaucoup plus petites, alternent avec eux et sont localisées vis-à-vis les cloisons: ce sont les pétales (Fig. 26).

Un peu au-dessous du point de départ des sépales, chacun des faisceaux *ds* se bifurque (Figs. 9, 24). Du côté interne part le dorsal du carpelle (*d*), et du côté externe un faisceau (*s*) destiné au sépale opposé. Ces deux faisceaux se voient d'abord côte à côte (Fig. 24), puis ils divergent. Le dorsal s'incurve vers le centre (Fig. 1), et continue sa course dans la partie supérieure de la paroi ovarienne pour monter ensuite dans un style. Quant au faisceau externe (*s*), il passe dans le sépale.

Les faisceaux *lps* se divisent aussi en un groupe de cordons vasculaires dont voici la course ultérieure (Figs. 9, 10, 25). Du côté interne se détachent deux faisceaux latéraux (*l*) destinés à des carpelles contigus. Ces faisceaux s'écartent latéralement, puis chacun d'eux, après s'être rattaché au dorsal par une anastomose, se rend au style ducarpelle auquel il appartient. Deux autres faisceaux (*s*), plus externes, passent à deux sépales voisins, où chacun d'eux se relie au cordon médian du sépale. Enfin un dernier faisceau (*p*) continue directement sa course et il entre dans le pétale.

Le mode d'origine des faisceaux qui prennent ici naissance diffère beaucoup de celui des faisceaux à la base de l'ovaire. L'émergence du dorsal (*d*) et du faisceau destiné au sépale (*s*) résulte de la bifurcation du cordon vasculaire *ds*. Parfois la division du faisceau *ds* est parfaitement tangentielle (Fig. 24): elle s'explique si l'on admet que le cordon *ds* représente un faisceau carpellaire et un faisceau du sépale, qu'il équivaut à leur base conjointe. La course du faisceau *lps* est à rapprocher de celle du faisceau *ds*, à cette différence près que fondamentalement une scission radiale accompagne la bifurcation tangentielle (Figs. 9, 10, 25). Situé à la limite théorique de deux carpelles, d'un pétale et de deux sépales, le cordon *lps* représente la base de tous les faisceaux qu'il émet à ces différentes pièces florales. Ce niveau n'est pas un noeud, mais la fin d'une concrescence — concrescence qui, chez les *Begonia*, se poursuit plus haut, jusqu'au sommet même de l'ovaire.

En même temps que surviennent ces changements dans la vascularisation de la paroi externe, les faisceaux *vv*, localisés près de chacun des placentas, commencent à se diviser radialement (Fig. 10). A la base de l'ovaire (Fig. 4), on a observé l'organisation de ces cordons vasculaires à partir de deux faisceaux ventraux (*v*) appartenant à des carpelles adjacents. Chaque ventral reprend maintenant son indépendance. Les deux ventraux issus d'un même faisceau *vv* s'écartent l'un de



FIGS. 15, 16 — Fig. 15. Fleur pistillée de l'*Hillebrandia sandwicensis*, vue du sommet. On remarque les sépales, les pétales (déjà fanés à ce moment), l'ouverture du sommet de l'ovaire, les styles et les stigmates. Les grandes bractées, qui persistent jusqu'après la déhiscence de la capsule, ont été enlevées. × 3.5. Fig. 16. Fleur de l'*Hillebrandia sandwicensis* vue de côté, et montrant la partie supérieure, libre, de l'ovaire. Noter aussi sépales, pétales, styles et stigmates. × 3.5.

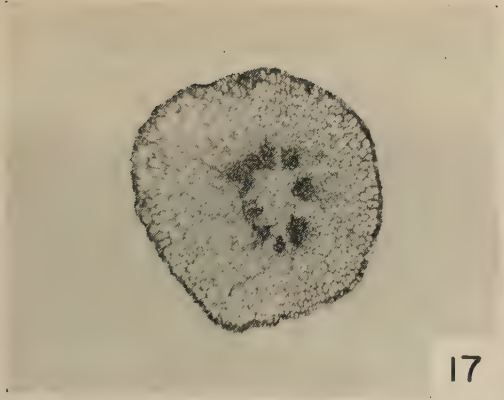
l'autre, laissant une zone parenchymateuse au milieu de la cloison, à la limite théorique des carpelles, et ils se dirigent vers les styles de deux carpelles voisins. Quelques ramifications mineures accompagnent parfois les ventraux.

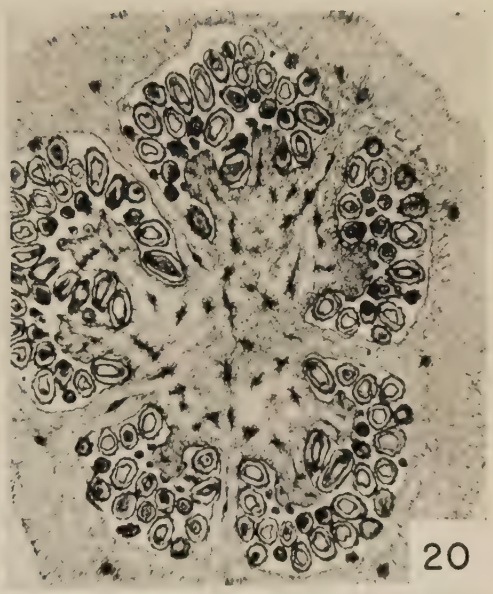
Sépales et pétales sont maintenant libres de l'ovaire (Figs. 11, 26). On voit, à l'extérieur, les cinq sépales, richement vascularisés. Ils correspondent au périanthe de la fleur des *Begonia*. Malgré les apparences (Fig. 15), ils ne se détachent pas tout à fait au même niveau: la préfloraison est en effet quinconciale, comme chez les *Begonia*, caractère qui ressort beaucoup plus nettement dans la fleur jeune. Les cinq pétales, minces languettes parenchymateuses, sont beaucoup plus petits que les sépales, et, comme c'est souvent le cas (Eames and MacDaniels, 1947), ils ne reçoivent qu'un faisceau (Fig. 26). Jeunes, ils paraissent concaves. Dans les plantes en culture au Jardin Botanique de Montréal, ils étaient déjà fanés au moment de l'ouverture des fleurs: c'est dans cet état qu'on peut les observer dans les figures 15 et 16, et aussi, en coupe, dans les figures 27 et 28. Pourtant, malgré leur faible dimension et leur brève durée, l'unique faisceau vasculaire qu'ils reçoivent se ramifie en trois branches parallèles. L'on est tenté d'y voir des organes en régression, et de penser que les pétales, absents chez les *Begonia*, sont en voie de disparition chez l'*Hillebrandia*.

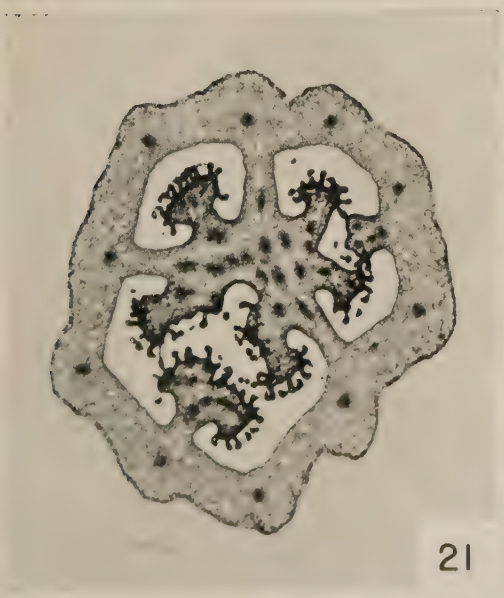
Il y a lieu de mentionner ici la présence de poils glandulaires (Irmscher, 1925;

→

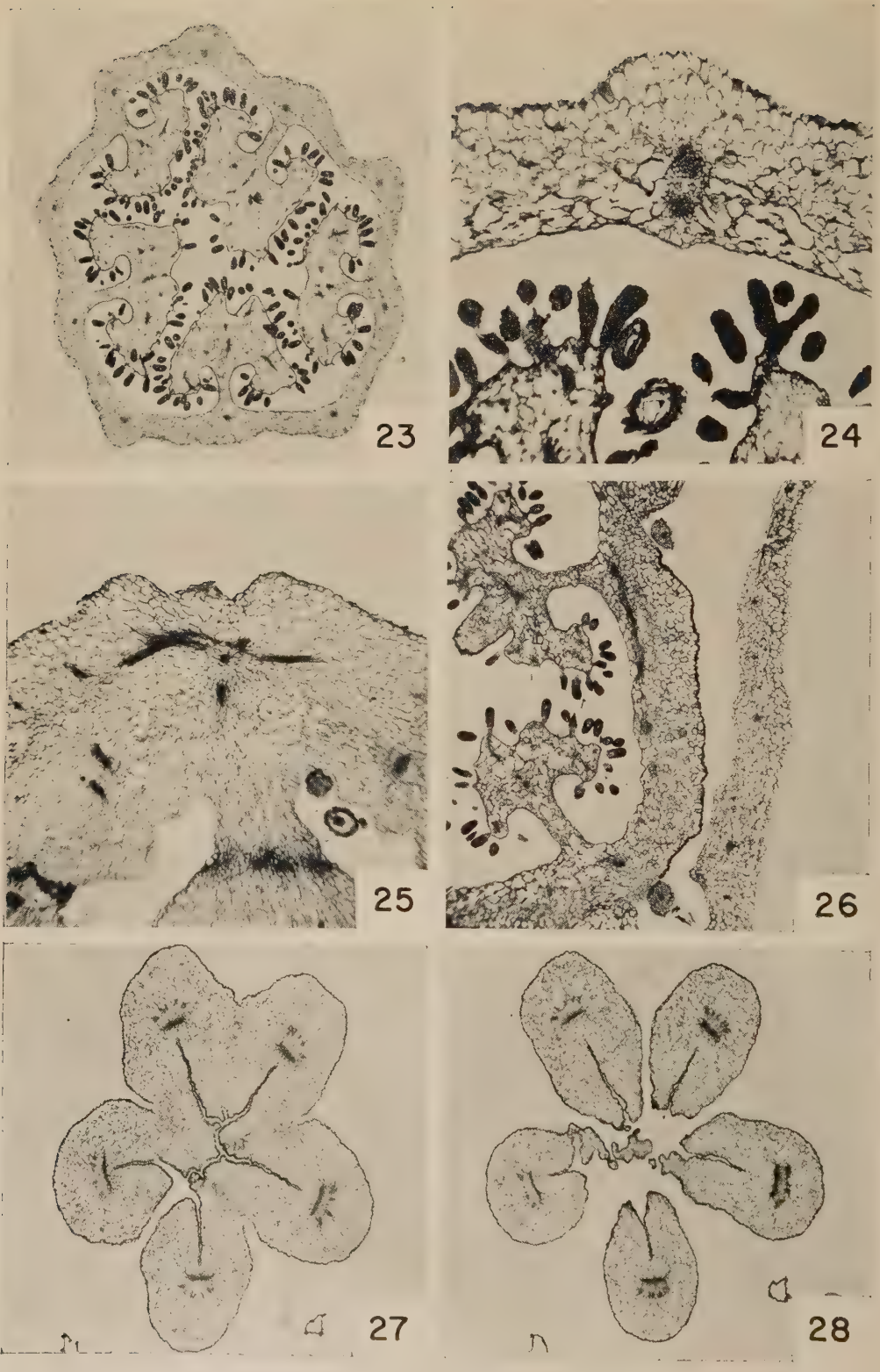
FIGS. 17-22 — Photomicrographies illustrant quelques-uns des stades représentés dans les figures 1-6. On a indiqué entre parenthèses les diagrammes correspondants. Fig. 17 (Diagramme 2). C.t. du pédicelle. × 20. Fig. 18 (Diagr. 3). Deux faisceaux *ds* quittent la stèle. × 17. Fig. 19 (Diagr. 4). C.t. vers le niveau où prennent naissance les faisceaux *vv* et *pl*. × 20. Fig. 20 (Diagr. 5). Région à placentation axile. × 18. Fig. 21 (Diagr. 6). Passage de la placentation axile à la placentation pariétale. Jeune fleur. × 25. Fig. 22 (Diagr. 6). Passage de la placentation axile à la placentation pariétale. Fleur plus âgée que la précédente. × 18.







FIGS. 17-22.



FIGS. 23-28.

Degener, 1938), parfois considérés comme des staminodes. Assez souvent visibles dans les coupes des fleurs provenant d'Hawaii, ces poils ne furent jamais observés sur les spécimens récoltés au Jardin Botanique de Montréal. On les remarque surtout à l'endroit où le périanthe se détache de l'ovaire. Bien qu'ils soient plus abondants près de la marge des sépales, ils paraissent distribués sans ordre défini, et l'on en observe parfois à l'extérieur des pétales. Ils ne reçoivent aucun faisceau vasculaire.

Le sommet de l'ovaire — Dégagé du périanthe, le sommet de l'ovaire laisse maintenant voir les faisceaux vasculaires répartis en cinq groupes (Figs. 11, 12, 27), c'est-à-dire que tous les faisceaux d'un même carpelle, quelle qu'ait été leur distribution à un niveau inférieur, sont rassemblés. Certains de ces faisceaux peuvent se ramifier: très généralement un cordon vasculaire prend ainsi naissance entre le latéral et le dorsal (Figs. 9, 10, 25). Les carpelles, bien distincts, apparaissent comme des structures condupliquées, unies latéralement. Les faisceaux y sont localisés loin de la marge (Figs. 13, 27). De la cavité ovarienne, graduellement recouverte par les cloisons épaissies, il ne subsiste qu'une étroite ouverture. Toutefois, si les marges de chaque carpelle se trouvent ainsi rapprochées, elles ne s'unissent pas: l'ovaire n'est pas complètement clos.

Ce caractère ne manque pas d'intérêt. En prenant les mots dans leur sens littéral, on pourrait dire que, dans l'*Hillebrandia*, l'angiospermie n'est pas tout à fait atteinte. Ce serait évidemment simplifier outre mesure la notion d'angiosperme que de la définir aussi sommairement (Eames, 1951). L'ouverture de l'ovaire au sommet a cependant son importance: c'est là sans doute un caractère primitif, que l'on retrouve dans quelques groupes. Il est bien connu chez le *Reseda*, où Hunt (1937) et Arber (1942) l'ont étudié. Eames (1951) mentionne un certain nombre de familles où il se rencontre. Bailey (1954) et ses collaborateurs l'ont mis en évidence en particulier dans les genres *Tetracentron* et *Trochodendron* (Nast & Bailey, 1945), où les carpelles sont soudés latéralement, et qui sont monotypiques comme le genre *Hillebrandia*. Si l'on admet que le pistil est formé de carpelles homologues de feuilles, l'ouverture du sommet de l'ovaire se comprend: le carpelle est ici comparable à une feuille repliée, mais sans qu'il y ait adhérence entre les deux épidermes ainsi rapprochés. Il y aura lieu de revenir sur cet important détail de structure.

Avec les figures 12, 13 et 27, on est parvenu au niveau des styles et la séparation s'amorce entre les carpelles. La cavité ovarienne est réduite à cinq fentes étroites, qui rayonnent à partir du centre, et qui constituent une sorte de canal étoilé, bordé par des cellules épidermiques bien distinctes. Chacune de ces fentes marque le plan médian d'un carpelle. Dans la figure 13, deux styles sont déjà entièrement détachés, tandis que les trois autres achèvent de le faire. Chaque carpelle acquiert ainsi son indépendance complète. La séparation a lieu au milieu même de la cloison formée par les parois de deux carpelles adjacents. Elle confirme le caractère double de cette cloison, déjà postulé à cause de sa position, de la nature des faisceaux *vv* et *lps* qui y montent, et de la valeur morphologique des placentas.

Figs. 23-28 - Photomicrographies illustrant quelques-uns des stades représentés dans les figures 7-14. On a indiqué entre parenthèses les diagrammes correspondants. Fig. 23 (Diagramme 7). C.t. dans la région à placentation pariétale. · 12. Fig. 24 (Diagr. 9). C.t. montrant le faisceau dorsal d'un carpelle et un faisceau d'un sépale, provenant de la scission tangentielle du faisceau *ds*. × 35. Fig. 25. (Diagr. 9 et 10). Scission tangentielle et radiale du faisceau *lps*. De l'extérieur à l'intérieur, le faisceau *lps* a donné deux faisceaux destinés à deux sépales différents, un faisceau d'un pétale, un faisceau qui représente les latéraux de deux carpelles voisins et qui se divisera radialement. × 28. Fig. 26 (Diagr. 11). C.t. montrant un sépale, richement vascularisé, et deux pétales, ainsi que la paroi ovarienne. · 17. Fig. 27 (Diagr. 12). Le sommet de l'ovaire, formé de cinq carpelles condupliqués. × 13. Fig. 28 (Diagr. 14). Au niveau des styles, les cinq carpelles indépendants. · 13. Au bas des figures 27 et 28, on voit deux pétales en c.t.

Dans l'angle du canal formé le long du style par le repli du carpelle, on distingue une zone de tissus qui retiennent fortement les colorants (Fig. 12, *tt*). Ces tissus débutent au sommet du stigmate, descendent dans chaque style entre les faisceaux et la zone épidermique, et débouchent dans la cavité ovarienne au voisinage des placentas (Figs. 1, 12, 13, 14, 27, 28). Ce sont des tissus de transmission (Arber, 1937, 1942) ou tissus stigmatoïdes (Esau, 1953), qui servent probablement à conduire les tubes polliniques aux ovules. Si, du point de vue histologique, l'ovaire n'est pas fermé, il paraît quand même physiologiquement clos. Aucune observation n'a cependant été faite sur le trajet du tube pollinique. Par ailleurs une étude plus complète pourrait fort bien révéler la présence occasionnelle de grains de pollen à l'intérieur de l'ovaire, comme on l'a rapporté d'abord dans le *Butomopsis* (Johri, 1936), et, plus récemment, dans un certain nombre d'autres taxa (Johri et Bhatnagar, 1957).[3]

Dans les figures 14 et 28, les cinq styles sont devenus indépendants. Les carpelles, complètement séparés les uns des autres, sont parfaitement distincts, et leur individualité comme pièces composantes de la fleur apparaît aussi nettement qu'on peut le désirer. Plus haut dans le style, le canal du carpelle se réduit à une légère indentation et finit par disparaître.

Telle est la course des faisceaux depuis la base de la fleur. Elle met fortement en relief l'individualité des carpelles, des pétales et des sépales, et cadre très bien avec l'interprétation de la fleur comme étant constituée de pièces unies latéralement et tangentiellement.

Conclusions

1. Dans l'opinion de l'auteur, la fleur pistillée de l'*Hillebrandia* est composée d'un pistil constitué de cinq carpelles, et d'un périanthe comprenant cinq pétales et cinq sépales. Périanthe et pistil sont unis sur une partie de leur longueur et forment un ovaire incomplètement infère de nature entièrement appendiculaire.

2. Cette interprétation rejoint celle qui a été proposée pour le genre *Begonia* (Gauthier, 1950). La structure de la fleur présente dans les deux genres les mêmes traits essentiels: les différences proviennent de l'adhérence plus ou moins complète du périanthe et du pistil, et de la soudure plus ou moins poussée des marges carpellaires. Ainsi la fleur de l'*Hillebrandia* permet de mieux comprendre celle des *Begonia*.

Dans l'*Hillebrandia*, pétales et sépales se détachent du pistil au-dessous du sommet de l'ovaire, et les scissions des faisceaux, qui marquent le départ des pièces florales, apparaissent dans la paroi externe au niveau de la cavité ovarienne. Il n'y a pas de noeud à cet endroit (Figs. 24, 25). Il n'y en a pas non plus dans la partie supérieure de la fleur, où les carpelles sont bien visibles (Figs. 27, 28). Chez les *Begonia*, les sépales se détachent au sommet même de l'ovaire et l'interprétation devient plus difficile. Toutefois, l'*Hillebrandia* montre qu'il s'agit dans l'un et l'autre cas de l'adhérence plus ou moins complète des carpelles et du périanthe: l'ovaire semi-infère de l'*Hillebrandia* représente une étape vers l'ovaire entièrement infère des *Begonia*.

Quant au pistil, dans les deux genres sa structure se comprend si on le considère comme formé de carpelles homologues de feuilles. Ainsi l'ovaire des *Begonia*, pluriloculaire et à placentation axile, et celui de l'*Hillebrandia*, uniloculaire et à placentation pariétale, deviennent comparables. Dans le genre *Begonia*, seule la course des faisceaux permet de déterminer la position des carpelles: les ailes de la fleur en indiquent la région médiane. Chez l'*Hillebrandia*, le sommet de l'ovaire est libre du périanthe, et les carpelles se distinguent avec une singulière netteté (Figs. 27, 28). Leur position confirme en tous points celle que la course des faisceaux avait permis d'établir dans le genre *Begonia*. L'interprétation proposée rend également compte de plusieurs détails de structure: la présence des deux types de placentation ainsi que l'ouverture au sommet de l'ovaire de l'*Hillebrandia*, les placentas simples et bifides et la communication

3. Depuis la rédaction de ce travail, l'auteur a observé des grains de pollen, dont quelques-uns étaient en germination, dans l'ouverture même du sommet de l'ovaire, au-dessous des styles.

entre les loges dans l'ovaire des *Begonia* dépendent de la fusion plus ou moins intime des marges carpellaires.

3. Il y a lieu de revenir sur un point: le lien fortement accusé entre carpelles adjacents. Plusieurs indices donnent à penser que, dans l'évolution de la fleur des *Begonia*, l'union entre carpelles voisins s'est opérée en premier lieu, et que les marges d'un même carpelle se sont fusionnées postérieurement au centre de l'ovaire. Si l'on accepte ce point de vue, on en vient à la conclusion que, dans ce groupe, la placentation pariétale a précédé la placentation axile. C'est l'idée que l'auteur avait émise (Gauthier, 1950, p. 63) et que paraît confirmer l'étude de l'*Hillebrandia*.

Précisons d'abord que nous n'avons jamais considéré la placentation pariétale comme étant toujours primitive par rapport à la placentation axile. Dans plusieurs cas (*Ranales*, etc.), il paraît clair que la fermeture à peu près complète du carpelle a précédé l'organisation du pistil composé. La placentation axile est alors réalisée d'emblée, et ce n'est que secondairement que l'on passe à un autre mode de placentation, par exemple à la placentation centrale (Caryophyllacées, Primulacées). Mais n'est-il pas possible que, dans d'autres groupes, l'union entre carpelles voisins soit survenue avant la fermeture des carpelles, et que l'ovaire clos ait pris naissance avant le carpelle clos? Dans l'origine phylogénétique d'un pistil de ce type, on peut concevoir qu'il y a d'abord union latérale des carpelles (Lawrence, 1951, pp. 76-77, Figs. 6, 7) en une sorte de collerette, puis de coupe ou de bouteille ouverte au sommet (*Hillebrandia* est à ce stade). C'est seulement ensuite qu'une fusion plus poussée entre les pièces composantes referme entièrement l'ovaire. Cette fusion peut ne survenir qu'au sommet, au niveau du style, entre l'extrémité déjà rapprochée des carpelles (*Hillebrandia* est tout près de ce stade): la placentation demeure pariétale et l'ovaire uniloculaire. Elle peut s'étendre à la région centrale de l'ovaire lui-même, et la placentation devient axile (*Hillebrandia* est au tout début de ce stade, *Begonia* à la fin: la fusion y est encore incomplète au sommet des loges ovariennes). Dans cette perspective, la formation de l'ovaire clos précède la fermeture du carpelle: c'est la placentation pariétale qui est primitive, et la placentation axile, au sens descriptif, n'apparaît que secondairement, lorsque les placentas ou les marges carpellaires se rejoignent au centre de l'ovaire. Selon le cas, les deux modes de placentation peuvent être primitifs. Quoi qu'il en soit, la fleur de l'*Hillebrandia*, où l'ouverture de l'ovaire au sommet (caractère incontestablement primitif) accompagne la placentation pariétale, apporte un argument de plus pour considérer que, dans les Bégoniacées, la placentation pariétale est primitive.

4. Dans son étude sur la placentation chez les Angiospermes, Puri (1952b) a formulé quelques observations sur lesquelles il n'est pas inopportun de revenir. Pour lui la notion habituelle, topographique, de la placentation n'est pas suffisamment précise. Il faut avoir recours à des critères plus rigoureux, basés surtout sur la disposition des faisceaux vasculaires. Dans la placentation axile, le placenta résulte de la fusion des deux marges d'un même carpelle et il est alimenté par les ventraux de ce seul carpelle. Les ventraux sont localisés sur un même rayon que le faisceau dorsal, et présentent l'inversion caractéristique du xylème et du phloème. Dans la placentation pariétale, le placenta est localisé à la marge de deux carpelles adjacents et reçoit les ventraux de ces deux carpelles; les cordons ventraux ne sont pas sur un même rayon que le dorsal et ils ne montrent aucune inversion. Lorsque ces caractères sont présents, la placentation doit être considérée comme pariétale, même si, comme il arrive occasionnellement, les ovules paraissent insérés sur une colonne au centre de l'ovaire.

Nul doute que dans le genre *Hillebrandia*, et dans les cas similaires, la placentation est fondamentalement pariétale. En d'autres termes, à la base de l'ovaire, la placentation est pseudo-axile, ou "faussement axile" (Parkin, 1955; Douglas, 1957). L'auteur est d'accord avec Puri sur ce point. Toutefois, pour désigner les modes de placentation, il semble préférable de conserver aux termes "axile" et "pariétal" le sens

purement descriptif qu'on leur attribue généralement (Lawrence, 1951). Autrement on risque d'enlever à ces termes toute valeur pratique.

Du point de vue morphologique, la distinction faite par Puri n'en reste pas moins intéressante. Elle met peut-être en relief deux modalités quelque peu différentes de parvenir à la formation du pistil composé. Au sens proposé par Puri, la placentation axile se rencontre lorsque le pistil résulte de l'union de carpelles préalablement fermés: l'individualité des carpelles est alors très marquée, et le squelette vasculaire l'indique fortement. Dans la placentation pariétale, la position comme la vascularisation des placentas mettent en relief le caractère primordial de la soudure entre carpelles voisins. Ce sont là d'ailleurs des cas extrêmes: tous les degrés d'adhérence peuvent exister aussi bien entre les marges d'un même carpelle qu'entre celles de carpelles adjacents (Eames, 1951).

5. La présence d'un ovaire ouvert au sommet et pourtant déjà partiellement infère permet de penser que, dans ce groupe, la fusion du périanthe et du pistil s'est produite de bonne heure, tout au moins avant la constitution d'un ovaire complètement clos. Dans l'évolution de la fleur des Angiospermes, on considère habituellement que la fermeture du carpelle s'est opérée en premier lieu; par cohérence de carpelles clos, on passe au pistil composé; enfin l'ovaire, d'abord supère, devient infère, soit par invagination du réceptable, soit, le plus souvent, par suite de la concrescence du pistil et du périanthe. Sans doute en a-t-il souvent été ainsi. Toutefois l'*Hillebrandia* porte à croire que, dans certains taxa du moins, la fermeture des carpelles et leur union avec le périanthe se sont accomplies simultanément, que l'adhérence du périanthe et de l'ovaire était en grande partie réalisée avant la fermeture des carpelles. Si l'on accepte cette idée, on sera moins surpris de voir apparaître tôt dans l'histoire des Angiospermes des familles à ovaire infère: en certains cas, au moment même où l'ovaire est devenu clos, il était déjà infère.

6. L'*Hillebrandia* présente quelques autres particularités intéressantes, mais sans rapport direct avec l'interprétation proposée de la fleur. Par exemple: les bractées persistantes; la déhiscence, qui se fait au sommet de la capsule. Il y a lieu cependant de mentionner que la paroi "ovarienne" comprend deux zones distinctes. La région interne est formée d'un parenchyme lacuneux, riche en chloroplastes, et qui, dans la coupe longitudinale d'une fleur fraîche, prend une teinte verte très prononcée. La zone externe, au contraire, est constituée d'un parenchyme plus compact, dépourvu de chloroplastes.

Dans l'hypothèse de la concrescence d'un périanthe et d'un pistil de nature appendiculaire, cette structure histologique peut avoir une signification: l'absence de chlorophylle à la périphérie est peut-être en rapport avec le fait que cette région correspond à la base du périanthe; sa présence dans la zone voisine de la cavité ovarienne marque l'emplacement du carpelle, qui généralement garde assez longtemps ce caractère foliaire. Pareille manière de voir ne manque pas d'attrait, mais seule une étude comparative plus approfondie pourrait en montrer le bien-fondé.

Summary

Hillebrandia is a monotypic genus of the family Begoniaceae. Its only species, *H. sandwicensis*, is endemic in the Hawaiian Archipelago. The pistillate flower differs from that found in most species of *Begonia* by some important characters, especially the combination of a semi-inferior ovary and open carpels. Flowers from both wild and cultivated material were studied.

The origin of the various bundles at the base of the ovary does not present any difficulty (Figs. 2, 3, 4), with the exception, perhaps, of the fused ventrals (*vv*). Yet it seems that these last mentioned bundles are produced by the fusion of separate ventral strands (Fig. 4, *v*). Many placental bundles (*pl*), which are branches of the ventrals, also begin at the same level. The uppermost point of the axis is shown by Fig. 4.

The various floral bundles are best seen in Fig. 5. There are ten bundles in the "ovary wall": five (*ds*), each represent-

ing one dorsal carpellary and one sepal bundle; five (*lps*), each representing two laterals of adjacent carpels, one petal and two sepal bundles. In the central part of the flower are seen five bundles (*vv*), each standing for fused ventrals of adjacent carpels, and a group of placental strands (*pl*). If the perianth is omitted, such a structure may be considered as an ovary made up of five coherent carpels. Each is homologous with a leaf and has the following bundles: one dorsal, two laterals (fused with laterals of adjacent carpels), and two ventrals (also fused with ventrals of adjacent carpels). At this level, placentation is axile, the term being used in a purely descriptive or topographical sense (Lawrence, 1951).

The transition from axile to parietal placentation (Figs. 6, 21, 22) takes place with very little change in the course of the bundles, and it is clear that the ovule-bearing column at the base of the ovary results from the fusion of the basal parts of the fleshy placentae developed at the margins of the five carpels.

The next important level is where the perianth is detached from the ovary. A little below this level (Figs. 9, 10, 24, 25), bundles *ds* and *lps* divide, the former giving rise to the dorsal of a carpel and a sepal bundle, the latter to two laterals of adjacent carpels, one petal, and two sepal bundles (of adjacent sepals). The dorsal carpellary (*d*) and sepal bundle (*s*) thus result from the splitting of bundle *ds*. This splitting may be perfectly tangential (Fig. 24), and is best understood if it is admitted that bundle *ds* represents both the dorsal carpellary and sepal bundles congenitally united. Except for the radial division that accompanies tangential splitting, the behaviour of bundle *lps* is fundamentally similar, and can also be explained by the fact that this bundle represents the lower part of all the bundles to which it gives rise.

The five sepals are well vascularized (Fig. 26) and are homologous with the perianth of *Begonia*. The five small petals receive only one trace, which branches into three bundles. In the cultivated material of *Hillebrandia*, the petals seemed already wilted when the flower opened. These structures are absent in *Begonia*.

The top of the ovary is free from the perianth (Figs. 11, 12, 13, 14, 16, 27, 28), and shows the five open carpels. The gynoecium thus appears to be made up of five conduplicate units fused laterally. Though the ovary is open from an histological point of view, it appears physiologically closed. The stigmas are well developed and stigmatoid tissue is present in the styles[4]

Hillebrandia helps in understanding the flower of *Begonia*. In *Hillebrandia* the perianth is detached below the top of the ovary. There is no node at this level (Figs. 24, 25), nor is there any at the top of the ovary where the five carpels are clearly distinct (Figs. 27, 28). Owing to the fact that the ovary is completely inferior, the condition in *Begonia* is more difficult to interpret, but a comparison with *Hillebrandia* indicates that it is a matter of more or less complete adnation between the perianth and the gynoecium.

In *Begonia* the general disposition of the flower and the course of the bundles permit the location of the carpels to be determined, their median part being in the plane of the wings. *Hillebrandia*, with the upper part of the ovary free from the perianth and unsealed carpels, adds still stronger evidence, and confirms the conclusions arrived at in the study of *Begonia*.

The association of open carpels with parietal placentation in *Hillebrandia* suggests that, in this group, parietal placentation is the primitive condition. This substantiates the idea put forth (Gauthier, 1950) that, in the evolution of the *Begonia* flower, union between adjacent carpels occurred first (*Hillebrandia* is near this stage), and that only afterwards were the carpel margins joined in the center of the ovary (*Hillebrandia* is at the beginning of this stage; *Begonia*, at the end: the union between carpel margins is often incomplete at the top of the loculi). In many taxa, of course, the gynoecium has been formed by the fusion of previously closed carpels, and in such cases axile placentation is primitive.

4. Since this was written, pollen grains (some of them germinating) have been observed in the opening at the top of the ovary, below the styles.

While using the terms axile and parietal in a purely descriptive sense (the use of these terms otherwise becomes impracticable), the writer thinks that Puri's proposed definitions (1952b) of placentation are interesting, and may, in some way, be related to two slightly different ways by which the compound gynoecium originated. At any rate it is agreed that in *Hillebrandia* placentation is fundamentally parietal.

The presence of open carpels associated with a semi-inferior ovary indicates that, in this group, adnation between the perianth and the ovary is already well under way before the ovary becomes closed. It may be that the usual sequence which we think of (closure of the carpel, union of closed carpels to form a compound gynoecium, ovary at first superior, and afterwards inferior) is not the only possible one. In some taxa closure of the ovary and adnation with the perianth may have progressed simultaneously and when closure of the ovary is obtained, the ovary may have been at the same time inferior.

Mention is made of the fact that, in the ovary wall, there is an external zone of rather closely packed parenchyma cells devoid of chlorophyll, and an inner zone of lacunar parenchyma with abundant chloroplasts. In a longitudinal section of a fresh flower, that inner greenish region is striking. Chloroplasts are also present in the septa and the placentae. Attention is drawn to this condition, but, in the absence of a more extensive comparative study, the writer does not emphasize too much its possible morphological implications.

The pistillate flower of *Hillebrandia* may be interpreted as made up of a gynoecium of five coherent carpels homologous with leaves, to which is adnate a perianth of five sepals and five petals. The gynoecium and perianth are united for a part of their length to form an ovary which is semi-inferior and entirely appendicular. Such an interpretation is similar to the one proposed for *Begonia*, the main differences between the two genera resulting from the degree of adnation between perianth and gynoecium and from the more or less complete fusion of carpel margins. If such an interpretation is admitted, the flower of *Begonia*, with axile placentation and a multilocular ovary, is comparable to that of *Hillebrandia*, with parietal placentation and a one-celled, five-lobed ovarian chamber. The presence of axile and parietal placentation and the open carpels in *Hillebrandia*, the presence of simple and bilamellate placentae and the openings at the top of the loculi of *Begonia* also receive an explanation.

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SOME OBSERVATIONS ON THE POST-FERTILIZATION DEVELOPMENT OF THE EMBRYO SAC OF *SANTALUM*

S. P. BHATNAGAR

Department of Botany, University of Delhi, Delhi 8, India

The study of the female gametophyte of *Santalum album* goes as far back as 1836 when Griffith observed the protrusion of the tip of the embryo sac beyond the ovule and its growth into the ovarian cavity towards the stylar canal. In a subsequent paper, he (Griffith, 1843) described the posterior elongation of the embryo sac into the placentum, thus acquiring a ↳-shaped outline. He also referred to the presence of persistent pollen tubes which adhere firmly to the tip of the embryo sac. This unusual growth of the apical as well as the basal end of the embryo sac of *Santalum album* was confirmed by Henfrey (1856). Strasburger (1885) also gave an account of the embryo sac of *S. album*, followed by more detailed studies by Iyengar (1937) and Rao (1942). Fagerlind (1948) made similar observations on *S. pyrularium*.

The most recent work on *Santalum* is that of Paliwal (1956). Some of his findings, viz. the protrusion of the tips of synergids into branched haustoria, formation of an upper (micropylar) endosperm haustorium, the occurrence of a composite endosperm by the fusion of endosperms of different embryo sacs, and the absence of a suspensor in the embryo,

appeared to be so unusual that I was led to a fresh study of *Santalum album*. Material of *S. freycinetianum* and *S. obtusifolium* was also available for comparison.

Embryo Sac — The embryo sac conforms to the Polygonum type. The tip of the embryo sac extends beyond the ovule into the ovarian cavity and grows towards the stylar canal. At the other end a chalazal caecum grows downwards into the placental column leaving the antipodal cells *in situ*. The outline of the mature embryo sac thus becomes ↳-shaped (Fig. 1).

Fertilization — Iyengar (1937) presumed that double fertilization does not occur in *S. album*. He stated: "The activities of the fusion-nucleus precede fertilization. The first sign of the activity becomes apparent at the time of the penetration of the pollen tube through the synergids. By the time the first male nucleus is discharged into the egg cell the first mitotic figure is seen in the fusion-nucleus. The second male nucleus remains as a disorganized mass in the pollen tube . . . By the time syngamy is completed, free endosperm nuclei are scattered in the bend of the embryo sac." This has already been contradicted by Paliwal (1956) who finds normal double fertilization. I have confirmed it in *S. freycinetianum*.

Synergid Haustoria — Paliwal (1956) emphasizes that in *S. album* and *S. yasi*, after fertilization, the wall of the embryo sac dissolves at the micropylar end and the tips of the synergids protrude beyond it, each giving rise to a branched haustorium (Fig. 3). He states that the synergid haustoria "... are thin and membranous like branched pollen tubes", and adds: "The synergid haustoria enter the tissue of the mamelon lying immediately above to protrude out at its tip into the stylar canal or its tissue." They disorganize only in later stages when both the endosperm and embryo are well advanced.

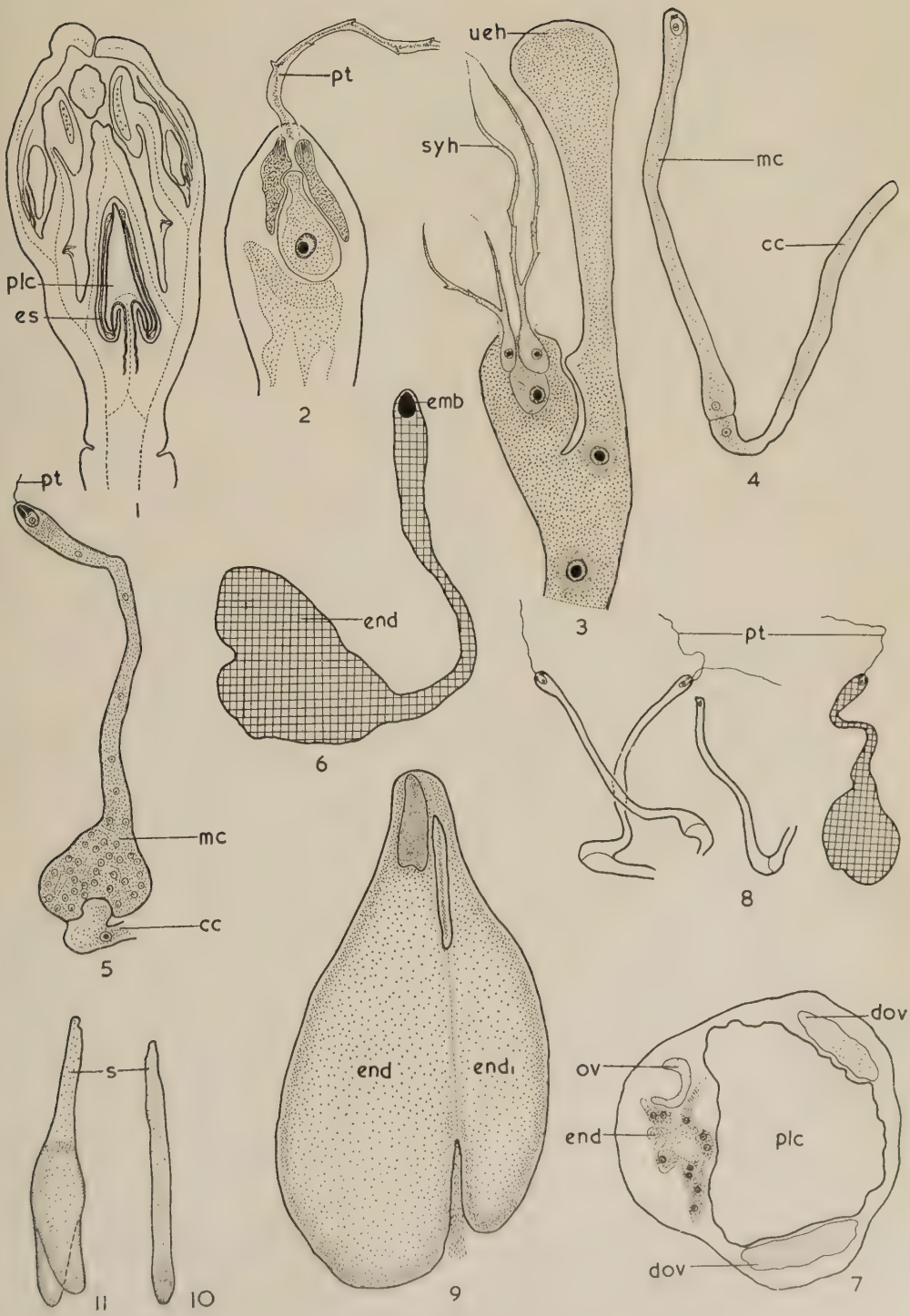
My preparations of *S. album* and *S. freycinetianum* show that in fact the synergids degenerate soon after fertilization (*see also* Iyengar, 1937; Rao, 1942). What have been interpreted as the synergid haustoria are the persistent pollen tubes which adhere to the embryo sac (Fig. 2). The pollen tubes can be seen quite easily in whole mounts of fertilized embryo sacs dissected out from the surrounding tissues. It stands to the credit of Griffith (1836, 1843) that he correctly interpreted these persistent tubular structures as pollen tubes.

Endosperm — The first division of the primary endosperm nucleus is followed by a transverse wall (Fig. 4). The lower chalazal chamber functions as the uninucleate chalazal haustorium, while the micropylar chamber, after many free nuclear divisions and cell formation (Figs. 5, 6), gives rise to the endosperm proper.

Paliwal (1956) states that the endosperm nuclei in the free arm of the embryo sac "... are arranged in a linear row closer to the outer wall of the embryo sac away from the mamelon. At this stage another lateral caecum arises from the upper part

→

Figs. 1-11 — *Santalum album*. Figs. 2-6, 8 and 11 from whole mounts of dissections; rest from microtome sections (*cc*, chalazal chamber; *dov*, degenerated ovule; *emb*, embryo; *end*, endosperm; *es*, embryo sac; *mc*, micropylar chamber; *ov*, ovule; *plc*, placental column; *pt*, pollen tube; *s*, suspensor; *syh*, synergid haustoria; *ueh*, upper endosperm haustorium). Fig. 1. L.s. flower to show position and outline of mature embryo sacs (diagrammatic). × 20. Fig. 2. Tip of embryo sac showing degenerated synergids, zygote and persistent pollen tube. × 297. Fig. 3. Paliwal's (1956) Fig. 86, showing the so-called synergid haustoria and upper endosperm haustorium. × 300. Fig. 4. Two-celled endosperm. × 50. Fig. 5. Upper part of embryo sac showing irregular distribution of endosperm nuclei in the micropylar chamber. × 50. Fig. 6. Lobed cellular endosperm. × 50. Fig. 7. T.s. ovary to show that endosperm develops in only one ovule, while the other two ovules are abortive (diagrammatic). × 83. Fig. 8. Four embryo sacs from the same ovary; endosperm has developed only in one of them. Note the thread-like pollen tubes. × 30. Fig. 9. Paliwal's (1956) Fig. 95, showing l.s. of "composite endosperm". Fig. 10. Embryo with well-developed suspensor. × 27. Fig. 11. Mature embryo with the two cotyledons. × 18.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FIGS. 1-11.

of the embryo sac not far below the zygote." This he designates as the upper endosperm haustorium (Fig. 3). He further adds: " One of the free endosperm nuclei comes to lie at the mouth of the caecum and seems to be responsible for the further growth of this structure, which is haustorial in function." It is said to destroy the tissues of the placentum above it, and in later stages it is cut off from the embryo sac due to the laying down of walls in the endosperm. In *S. yasi*, according to him, the upper endosperm haustorium is less aggressive.

I find that in *S. album* as well as in *S. freycinetianum* the endosperm nuclei are irregularly disposed (Fig. 5) in the micropylar chamber. A lateral micropylar extension may arise in rare cases but has no active haustorial function. In *S. obtusifolium* such a prolongation sometimes arises even before fertilization. Iyengar (1937) pointed out that wall formation in the endosperm starts from the apex downwards. In my preparations, on the other hand, walls are first laid down in the bulla (swollen basal portion of the micropylar chamber) and then progress upwards (*see also* Paliwal, 1956).

Nuclear divisions in the micropylar chamber are sometimes immediately followed by walls resulting in three or four cells. Free nuclear divisions then occur in each of these cells and they bulge out prominently giving a lobed appearance (Fig. 6). Later, wall formation is resumed in the coenocytic cells and the development progresses normally.

Paliwal (1956) claims that the endosperm of *Santalum* is a ' composite ' structure (Fig. 9), a feature so far known only in the Loranthaceae. I find that the endosperm has a concavo-convex form and surrounds the placental column on the concave side. In a superficial section it gives the appearance of two closely situated lobes. Longitudinal sections through the middle region of the endosperm show the placental column between these two lobes. This can give the false impression of two masses of endosperm (one on either side of the mamelon) fusing with each other. Paliwal's Fig. 95, reproduced here as Fig. 9, seems to have been drawn from such a section.

The development of the endosperm may be initiated in more than one embryo sac in an ovary, but it reaches maturity in only one of them. The remaining two embryo sacs invariably degenerate (Figs. 7, 8).

Embryo—According to Schacht (1866) and Strasburger (1878), the suspensor is short and cylindrical. Iyengar (1937) also reported a short and multicelled suspensor, while Rao (1942) states that it is only one-celled. Paliwal (1956) emphasized the absence of a suspensor. My preparations of both *S. album* and *S. freycinetianum* show a well developed suspensor (Figs. 10, 11).

Summary

The so-called synergid haustoria previously reported in *Santalum* are really the persistent pollen tubes. The endosperm does not produce a micropylar haustorium nor is there any composite endosperm. Endosperm develops in only one of the embryo sacs in an ovary. The embryo possesses a conspicuous suspensor.

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DEVELOPMENT OF THE BRACKEN FERN, *PTERIDIUM AQUILINUM* (L.) KUHN.—II. STELAR ONTOGENY OF THE SPORELING

JOAN E. GOTTLIEB
California Western University, San Diego 6, California, U.S.A.

Introduction

Since the turn of the century, much botanical interest has centered around the vascular tissues which are the "backbone" of all typical land plants. A rather cumbersome nomenclature has consequently been built up, some of the names redundant, superfluous, and even erroneous, but many quite convenient and apt. A number of attempts have been made to review and standardize the terminology (Nast, 1944; Smith, 1955), but the complex ferns have never been handled in this way, and it is rare for two authors to agree on the same name. To facilitate a description of the *Pteridium* stele to follow, a short background of stelar terminology is included at this point.

Our understanding of vascular anatomy has come a long way since DeBary (1877) first postulated the *fibrovascular bundle* as the fundamental conducting unit of the plant body. He recognized *collateral* bundles (xylem with an arc of phloem on the outside), *bicollateral* bundles (xylem with phloem on both sides), and *concentric* bundles (xylem surrounded completely by phloem). DeBary described two types of concentric bundles which are now termed *amphicribral* and *amphivasal*, depending respectively on whether the phloem surrounds the xylem or the xylem surrounds the phloem.

In 1886, Van Tieghem and Douliot validly proposed the *stele* or central cylinder as the fundamental unit of the vascular system and suggested that the primitive stele consists of a single fibrovascular strand (a concentric bundle of DeBary). They regarded the *endodermis* as the innermost layer of the cortex and the *pericycle* as the outermost layer or layers of the stele, although the origin of these tissues is still in dispute. However, their concept of a unified, coherent, central cylinder was a major contribution to subsequent work.

In 1899, Jeffrey reinterpreted the stelar theory and placed it on its modern phylogenetic basis. He proposed that the primitive type of stele consists of a central mass of xylem surrounded by phloem—a *protostele*. Subsequent writers have divided the protostele into three types—the *simple protostele* (or haplostele—Brebner, 1902) with a circular core of xylem

externally surrounded by a band of phloem; the *actinostele* (Brebner, 1902) which has a star-shaped xylem mass and peripheral phloem, but the phloem is most extensive in the bays between the xylem arms; and the *plectostele* (Zimmerman, 1930), in which the xylem and phloem alternate in ribbon-like bands.

A poorly understood trend in the ontogeny and phylogeny of the shoot of vascular plants has brought about an increase in the surface ratio of living cells to nonliving conducting elements (Bower, 1930). Even simple protosteles may have a " mixed xylem " with parenchyma intermingled among the tracheids. The derived actinostele and plectostele represent increasing invasion of the xylem by pericyclic parenchyma and phloem. Further stelar parenchymatization has produced steles with a tubular vascular region and a parenchymatous medulla or pith. The derivation of such *siphonosteles* (Jeffrey, 1899) from protosteles will be discussed in some detail later in this paper. According to Jeffrey, siphonosteles may be either *ectophloic* (phloem surrounding the xylem externally) or *amphiphloic* (phloem on both sides of the xylem). Except for the root steles of certain vascular plants and the shoot axes of lycopsids and psilopsids, the attainment of siphonostely is associated with the occurrence of *leaf gaps* or areas in the stele above a leaf trace which remain parenchymatous. Therefore, amphiphloic siphonosteles are further distinguished as *solenosteles* (Gwynne-Vaughan, 1901) if there is only one leaf gap in any cross-section and *dictyosteles* (Brebner, 1902) if there are " overlapping " leaf gaps. For steles with parenchymatous gaps other than foliar gaps, the term *perforated* has been proposed (Bower, 1923). The individual strands or " bundles " of vascular tissue seen in cross-sections of dictyosteles or more complex derivatives are called *meristeles* (Brebner, 1902) and may be taken as the equivalent of DeBary's fibrovascular bundles.

Thus far, the terms outlined are applicable only to the vascular cryptogams. In these, the vascular tissue must be regarded as a single unit. In spite of its often broken appearance in cross-section, it must be visualized in three dimensions as a continuous unit — a meshwork of vascular tissue and leaf gap parenchyma. In the higher plants, however, the influence of the leaves appears to be so much greater that vascular tissue is thought by some botanists to differentiate only in relation to the foliar primordia, and as a result, true interfascicular parenchyma is formed which is not clearly distinguishable from leaf gaps. For this type of stele, Brebner (1902) has coined the term *eustele*, an obvious misnomer, but one which is too firmly entrenched in the literature to replace. This type of central cylinder is further complicated and often obscured in perennial or arborescent plants by secondary activity.

Some dicotyledons and many monocotyledons have highly evolved *atactosteles* (Brebner, 1902) or literally " steles without arrangement ". Like the eustele, vascular bundles apparently differentiate only in relation to leaf influence, but they are scattered through the stem and not arranged in a neat ring.

Since the early 1900's when the stelar theory was first interpreted on a phylogenetic level, many attempts have been made to understand the vascular patterns of those ferns which have complex, concentric steles. These forms were apparently evolved polyphyletically, occurring today in five separate families — Matoniaceae (*Matonia*: Seward, 1899; Tansley & Lulham, 1905; Tansley, 1908), Cyatheaceae (*Cyathea*: Tansley & Lulham, 1904, *Alsophila*: Bower, 1923), Dicksoniaceae (*Cibotium*: Bower, 1923), Marattiaceae (*Marattia*: Charles, 1911; West, 1917, *Danaea*: Brebner, 1902), and Polypodiaceae (*Pteridium*, *Pteris*, some species of *Dennstaedtia*, *Platycerium*, etc.: Gwynne-Vaughan, 1903, 1905; Bower, 1923; Jeffrey, 1902). Posthumus (1924) has reviewed the literature on this subject. Morphogenetic studies of these diverse steles may ultimately shed important light on their phylogenetic derivation and meaning.

The cross-section of the adult subterranean stem of *Pteridium* is a familiar illustration in elementary texts. With minor deviations among the different varieties or subspecies, the striking vascular system of this fern consists of an outer circle of

bundles, the uppermost (in relation to the soil surface) being elongate, and an inner system of two similarly elongate bundles lying parallel to the large outer one. Each bundle, regardless of size, is completely delimited by a layer of black staining endodermal cells and is amphicribral in structure. The xylem core is mesarch and contains some parenchyma dispersed among the conducting elements. It has been demonstrated (Russow, 1873; Bliss, 1939) that the scalariform pitted metaxylem cells lack pit membranes on the overlapping end walls, and so may be classified as vessel elements. Two sclerenchyma bands separate the inner and outer vascular systems dorsally and ventrally, and another sclerenchymatous rim borders the stem periphery. The rest of the stem is composed of storage parenchyma. Several detailed analyses of this unusual stem exist, the most recent being that of Webster (1957). She has described the stele as a " perforated amphiphloic siphonostele with medullary bundles ", and this phrase, albeit a bit cumbersome, will be retained in this discussion because it paints an accurate verbal picture of its appearance and derivation.

One aspect of stelar theory which has been of considerable interest is the general rule that fern sporelings follow a basic, ontogenetic stelar sequence, starting with a simple, solid, conducting core, and developing characteristic derived types in most cases. A short summary of this path follows. It should be borne in mind that some of these stages are often indistinct or telescoped in individual ferns.

1. A protostelic tract forms the central axis at the base of the young plant, giving off the early sporeling leaves. (A few ferns, e.g. *Lygodium*, some species of *Gleichenia*, *Hymenophyllum*, retain this protostele into adulthood.)

2. A medullated stage follows at which level the center of the stele is occupied by parenchyma and the first phloem elements appear external to the xylem ring. The xylem ring is generally interrupted by leaf gaps. (Ectophloic siphonostele of *Ophioglossum*, *Helminthostachys*, *Schizaea*.)

3. Internal phloem and an internal endodermis appear, becoming continuous with the corresponding external tissues around the margins of the leaf gaps[1].

4. Failure of the axis to elongate produces a crowding of leaves, causing overlapping of the foliar gaps. (Dictyostele of *Asplenium*, *Athyrium*, *Dryopteris*, etc.).

In order to include *Pteridium* and other complex ferns, certain steps will have to be added to this classical scheme, and it is the aim of this paper to do this and thereby suggest their possible path of evolution from the more generalized types. A study of the general development of the bracken sporeling has already been presented (Gottlieb, 1958). In this paper, the stelar morphology will be traced in large part ontogenetically, with some reference to its morphogenetic significance. Such a study will help to provide a better interpretation of adult structure by tracing its development in the individual.

Materials and Methods

All sporelings used in this study were raised on soil according to a method described previously (Gottlieb, 1958).

Most of the slides used for the ontogenetic stelar descriptions were permanent mounts prepared by the following schedule, all solutions, stains, etc., being made up exactly according to specifications outlined in Johansen (1940) and Foster & Gifford (1947). Material was cut into maximum lengths of one centimeter, killed and fixed in formalin-aceto-alcohol (FAA), washed in 50 per cent alcohol, run up through a graded series of tertiary-butyl-alcohol (TBA), and infiltrated with and embedded in Fisher's Tissuemat, 54°-56°C. It was sectioned at ten microns or thicker, affixed to glass slides with a combination of Haupt's gelatin adhesive and 2 per cent formalin, and stained with 1 per cent safranin (4 to 24 hours) and 0·5 per cent fast green (1 to 5 minutes).

In developing a three-dimensional picture of stelar changes during sporeling

1. There has been considerable controversy over the ontogenetic order of stages 2 and 3. The acceptance here of ectophloic siphonostely ahead of amphiphloic siphonostely is based upon the author's work with sporelings of *Pteridium* and *Phlebodium*. This will be discussed later in this article. For the historical background of the problem, see Smith (1955, Vol. II, pp. 145-146).

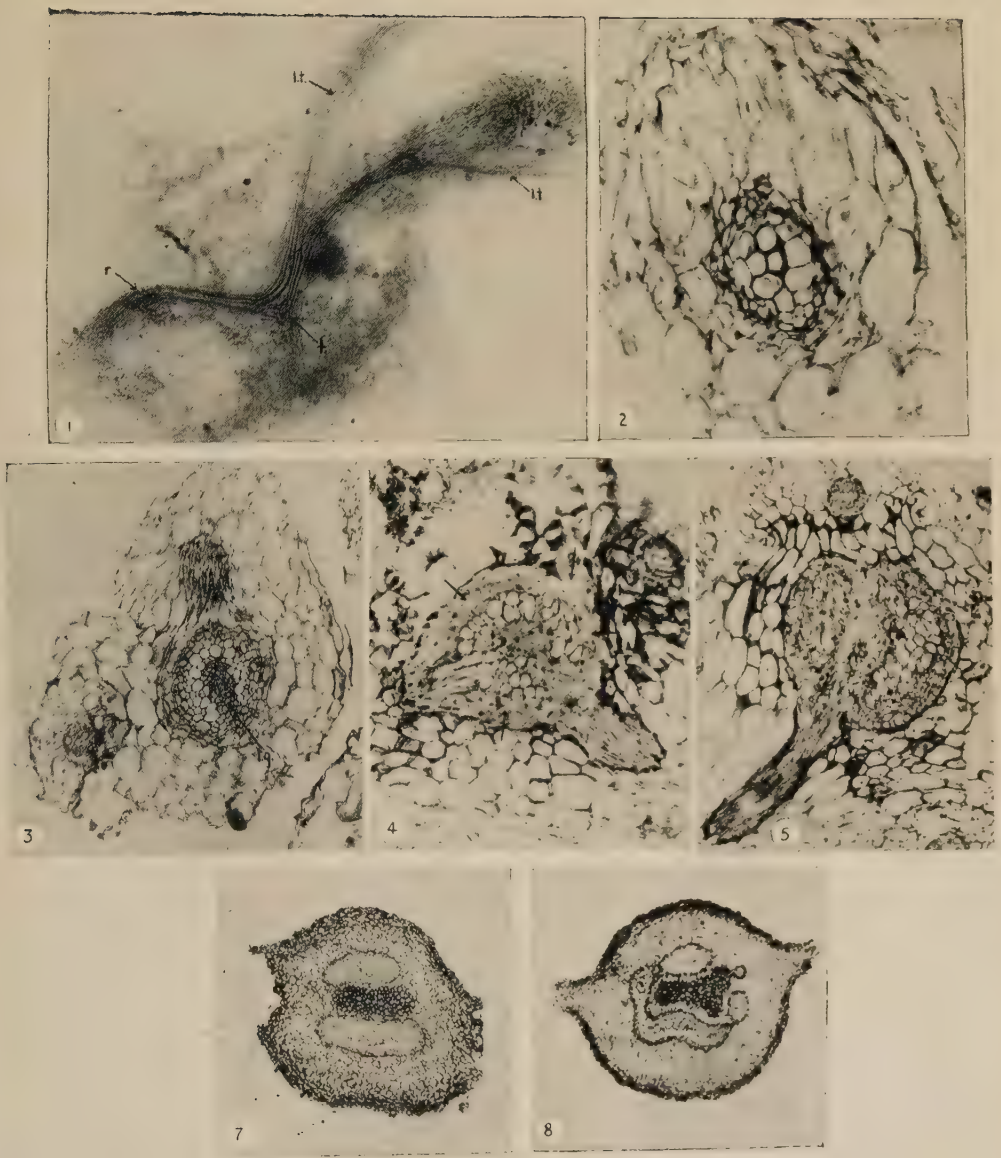
ontogeny, cleared and stained whole mounts of stems were found to be valuable supplements to microtome sections. A combination of basic fuchsin and sodium hydroxide was used according to the schedule described in Johansen (1940). After staining with the basic fuchsin, the stems were washed and immersed in a low concentration of NaOH (2 to 7 per cent, depending on the size and fragility of the material), and incubated at 40°-60°C. until colorless. A graded series of alcohol changes was used to dehydrate the material and during the first change of absolute alcohol, a few drops of glacial acetic acid were added to "turn the dye." Opaque cortical material was teased away under a dissecting microscope and the red stained vascular skeletons were mounted in damar on glass slides.

Observations

1. The Protostelic and Siphonostelic Stages — *Pteridium*, like all vascular cryptogams, begins as a sporeling with a protostelic stem. The first two or three leaf traces are connected to this solid vascular core and the protostelic tract runs for about 0·8 mm. Whole sporelings, stained and cleared, offer the best picture of the vascular system at this early stage (Fig. 1). Procambial tissue, and later, mature vascular tissue, both differentiate acropetally into the first leaf and root from an interesting transition zone where a few elements angle into the foot. No potential or mature vascular tissue has ever been observed to enter the gametophyte tissue.

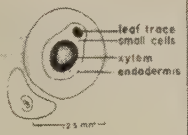
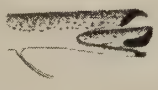
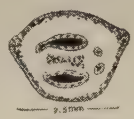
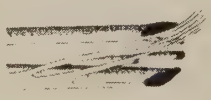
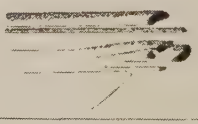
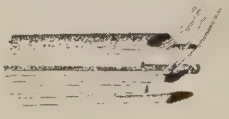
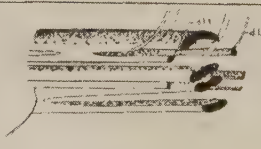
As the first leaf elongates, the stem becomes visible and possesses the narrow cauline vascular system described above. All bracken roots are diarch, from the primary root of the sporeling to the brittle, adventitious roots of the adult rhizome. The protostele of the sporeling stem with one or two leaves (Fig. 2) is clearly discernible in cross-section as a group of about twelve, large, procambial cells, but the first mature tracheids are noted in the basal part of the sporeling when three or four leaves have been formed. Potential xylem tissue (as xylem mother cells) is present, however, as the sporeling emerges from the archegonial chamber. In contrast, the phloem tissue appears and differentiates somewhat later. Lacmoid staining (Cheadle *et al.*, 1953) of material sectioned longitudinally and transversely revealed blue-staining callose granules in maturing phloem cells of the stele only above the fifth leaf; never have incipient or mature sieve cells been detected by the method below this level. Thus, phloem tissue does not appear to be present at all in the extreme basal portion of the young sporeling, but because of the limitations imposed by the single technique used, the exact stage at which the first phloem differentiates remains to be established.

As was mentioned in an earlier paper (Gottlieb, 1958), there is a considerable time gap between the appearance of leaf two and leaf three, during which the stem elongates considerably. Correlated with these external happenings, the stele internally is undergoing medullation leading to siphonostely. A cross-section at the level of the third or fourth leaf reveals a small cluster of parenchyma cells (pith) in the center of the stele (Fig. 3). Gaps are generally produced in the stele starting with the fourth leaf and the leaf traces are at this point crescent-shaped, with the open arms directed toward the main axis of the stem (Table 1). At this time also the endodermis begins to acquire the black staining reaction which is characteristic of that layer in the adult, suggesting an abundance of phenolic materials, perhaps tanniferous (Fig. 4). At the five-leaf stage, sieve elements are clearly visible as a discontinuous band encircling the xylem ring inside the endodermis. Thus, the vascular cylinder may be called an *ectophloic siphonostele* at this stage. Leaves six to eight are produced in rather rapid succession, and since they arise at 180° angles alternately around the stem, the effect of the overlapping leaf gaps is to break the vascular tube into two symmetrical bands (Fig. 5). It may be recalled that the stem is still growing vertically upward. A few phloem elements are seen on the inside of the xylem at this time, and soon the endodermis appears internally around the leaf gaps. Thus, just before rhizomes are established,



FIGS. 1-5, 7, 8 — Fig. 1. A two-leaf *Pteridium* sporeling, stained and cleared to show the initial protostele, the primary root (*r*), the simple leaf traces (*l.t.*), and area of attachment to the parent gametophyte (*f*). × 50. Fig. 2. T.s. of protostelic shoot of a two-leaf sporeling. Lignification is just beginning. × 189. Fig. 3. T.s. of the siphonostelic shoot of a four-leaf sporeling. A root trace is to be seen. Note the leaf gap (*l.g.*). × 63. Fig. 4. T.s. of a six-leaf sporeling, showing leaf and root trace connections with the ectophloic siphonostele, developing phloem (arrows) and a dark staining endodermal layer. × 63. Fig. 5. T.s. of an eight-leaf sporeling, showing the overlapping gaps of the alternate leaves dividing the stele into two strands. × 63. Fig. 7. T.s. of a *Pteridium* rhizome shortly after its formation. The two-strand vascular cylinder is distinct as is the sclerenchymatous pith. × 13. Fig. 8. T.s. of a rhizome at about 3·5 cm length, showing the first stelar perforations. × 13.

TABLE 1

growth stage	stem in cross section	stereodiagram of xylem	comments
upright leaves 1-2			one week old plants protostelic stem, ca 8mm. simple leaf traces; no gaps.
upright, leaves 3-5			five week old plants. pith appears in stele - ectophloic siphonostele. leaf traces crescent shaped; gaps left in stele. endodermis becomes tanniniferous. first mature phloem external to xylem
upright, leaves 6-8			two month old plants. leaf gaps overlap, producing an amphiphloic dictyostele. internal phloem visible, endodermis continuous externally; encroaching internally.
rhizomatous, 2-10 mm.			three month old plants, dorsi-ventral two-strand stele. pith becomes sclerenchymatous. leaf traces basically C-shaped, ends reflexed.
rhizomatous, perforation of dictyostele 1.6 cm.			three month old plants. perforation of stele begins. first short shoots appear.
rhizomatous, first short shoot 2 cm.			three month old plants leaves retain a direct vascular connection with rhizome
rhizomatous, origin first medullary bundle 2.2 cm.			3½-4 month old plants.
rhizomatous, first medullary bundle stage 5 cm.			four month old plants. adult petiolar pattern attained.
rhizomatous, second med. bundle stage 20 cm.			six months - one year plants. sclerenchyma in two bands separating peripheral from medullary systems direct leaf connections retained for some time into adulthood. (d.l.t.)

the stele (in transverse section) has the appearance of two complete and separate bundles, amphicribral in nature. However, it should more accurately be called an *amphiphloic siphonostele* with two gaps in every section or an *amphiphloic dictyostele.*

During the upright stage, the broadest dimension of the shoot apex is generally along the plane of leaf formation (plane bisecting the shoot apex and leaf primordia) (Fig. 6a, *l.p.-l.p.*) and since the alternate leaf gaps (*l.g.* 5 and *l.g.* 6) are responsible for interrupting the continuity of the vascular tube, the two resultant " bundles " (*x* and *y*) lie at right angles to the plane of leaf formation. The vascular traces for the seventh and eighth fronds, however, are formed approximately at right angles to those of the previous leaves (Fig. 6b, *l.p.-l.p.*). Leaf gaps seven and eight thus break the continuity of the cauline strands *x* and *y* and bundles *w* and *z* are formed at right angles by the joining of the separated ends of *x* and *y*. This directly precedes the equal dichotomy of the shoot apex which typically occurs after eight leaves have been produced. The plane of branching is thus parallel to the earlier plane of leaf formation (Fig. 6a, *br.-br.*) but is roughly perpendicular to the plane of formation of the last two leaves (Fig. 6b, *br.-br.*). From close observation of sporelings at this stage, it appears that the primordia of seventh and eighth leaf are formed at virtually the same time that the shoot apex divides. Thus, the foliar primordia may be displaced on the apical mound by the larger, new shoot apices. The mechanical and

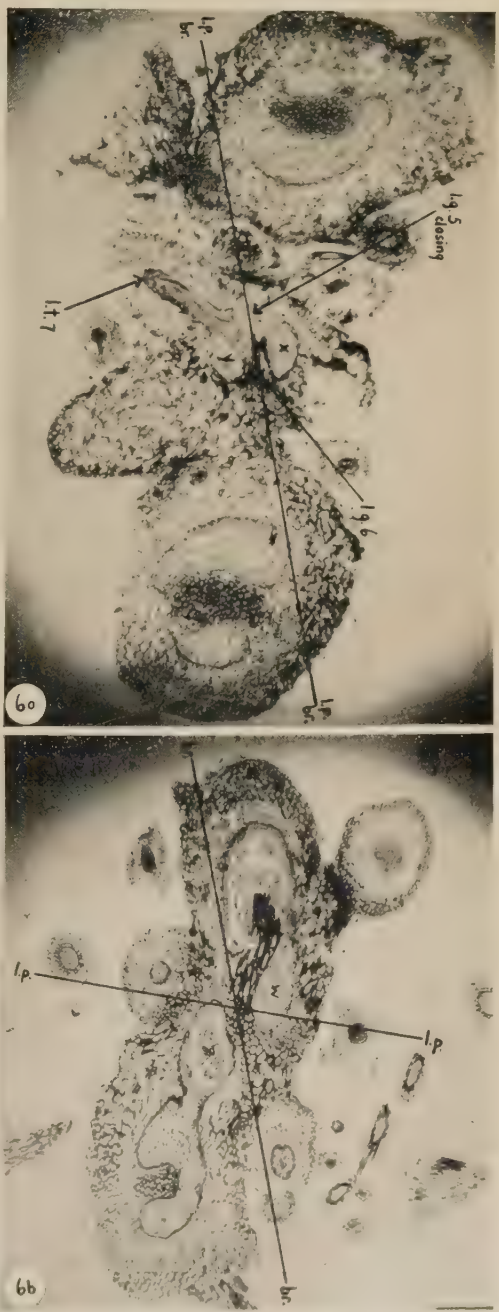


Fig. 6a, b.

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Fig. 6a, b — (*br.-br.*, plane of branching; *l.g.*, leaf gap; *l.p.-l.p.*, plane of leaf formation; *l.t.*, leaf trace; *x* and *y*, bundles of the upright axis). Fig. 6a. T.s. of the two *Pteridium* rhizomes shortly after their establishment. Note the mirror imagery of the vascular system; the smaller bundle is always uppermost with respect to the soil surface. The upright stem appears in cross-section at the level of the sixth leaf trace. × 16. Fig. 6b. T.s. of the *Pteridium* shoot system at the level of the dichotomy of the upright axis. The planes of leaf formation and branching are indicated for comparison with figure 6a. Bundles *w* and *z* of the upright axis may be seen entering respectively the two rhizome branches. × 16.

physiological mechanisms underlying this interesting transition stage need much additional work.

As shown in Fig. 6b, one of the two vascular strands of the upright axis continues into the rhizome produced on its side, and the other becomes continuous with the vascular system of the other rhizome. In the newly established rhizomes, the vascular strands expand considerably, so that the steles are C-shaped, the original strands from the upright axis (*w* and *z*) being continuous with only the morphologically lower sides of them.

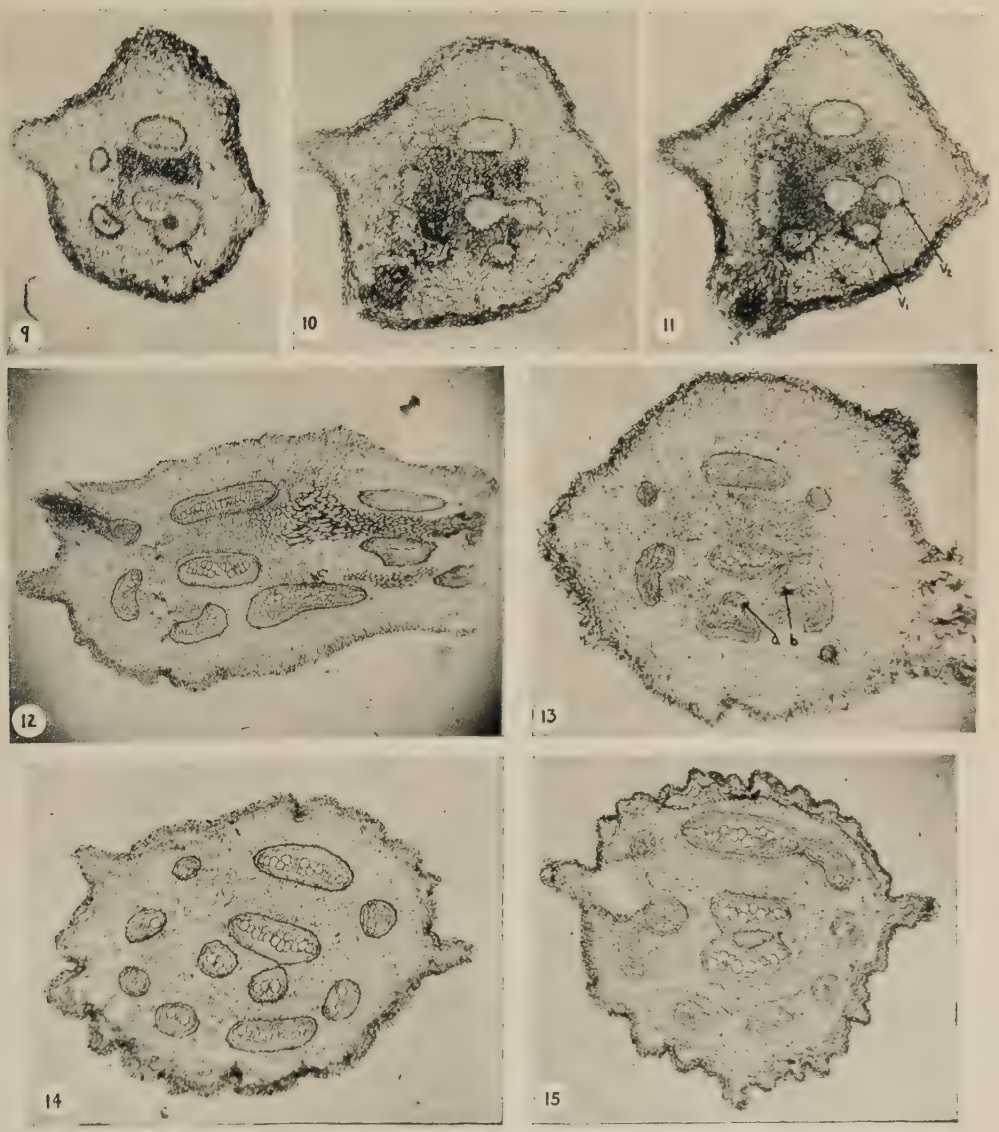
Developmentally, the picture may be described as follows. After forking, each of the new shoot apices produces a C-shaped stele, essentially restoring the whole vascular cylinder (minus one small segment), half of which was lost to its sister rhizome. The gap in this cylinder may be due to the influence of the branching or more likely to the leaf gap of the frond first produced on the rhizome, but this is not entirely clear. In any event, it is invariably a C-shped stele which is first seen in the newly formed rhizomes. Soon another leaf gap (related to the formation of the second leaf on the rhizome) appears on the side opposite this open end, and the *two-strand dictyostele* is restored. This time, however, the bundles have a distinct orientation, one being uppermost in relation to the soil surface and the other lowermost, although in other respects the stele is still a dictyostele, with only two leaf gaps in any one section from the alternating leaves. The leaf gaps are long at this stage, but occasionally one of them closes before the next leaf trace is formed on that side, and the true tubular nature of the vascular cylinder is revealed (Fig. 6b). The figures show the appearance of the adventitious roots and leaf petioles in cross-section at this level. The roots still have diarch protosteles, and the leaves now have the C-shaped petiole strands with the open arms of the vascular tissue recurved (directed away from the stem) as shown in Table 1.

As soon as the rhizomes are established, the pith of the stele differentiates as sclerenchyma, and a rod of thick-walled cells is now seen in the center of the stem (Fig. 7). Leaves, and later short shoots, arising alternately on the stem, perpetuate this two-strand stage until about 2 cm of rhizome have been formed.

Before discussing the stages leading up to the adult type complex stele, it might be à propos to make a few preliminary comments. The changes in the form of the cauline conducting system so far may be correlated easily with the changes in the appendages borne on the stem. When the first pinnate leaves of some size are formed, pith appears in the stele and leaf gaps form above the leaf traces, although it is by no means implied that these are causally related. As the internodes fail to elongate, the stele is broken into two strands. However, the changes beyond this point are somewhat more difficult to interpret and are not coincident with any external changes in the shoot morphology. The dissections of the stele up to now have all been foliar or ramular gaps, but now the gaps arise independently and must be called *perforations*.

2. Perforation and the Establishment of the Medullary System — The first evidence of stelar perforation is the separation laterally of small bundles from the flanks of the lower vascular band (Fig. 8), so that the stele comes to have as many as five or six masses of conducting tissue as seen in transverse section, each a complete amphicribral bundle, and all disposed in a ring with the original two bands still the largest. At this point the rhizomes are five or six centimeters in length and the original lowermost bundle expands inwardly, encroaching upon the pith and separating off from its inner surface a continuing portion of vascular tissue which comes to resemble a rod, the first part of the internal system. This medullary bundle (Figs. 9, 10, 11) anastomoses with the peripheral bundles at intervals and contributes to the vascularization of each short shoot at its origin, but it is separated from the peripheral system for the most part by a rod of sclerenchyma which differentiates between it and its parent lower bundle. Van Tieghem (1891) visualized the complex vascular system as a "polystele" built up from repeated dichotomy of a central "monostele". He postulated that

the medullary system and the peripheral bundles derived. Jeffrey (1899, 1917) was thus original correctly demonstrated that exactly the reverse is true, the inner strands originating from the outer ones.



FIGS. 9-15 — Figs. 9-11. A series of closely successive t.s. through a rhizome at about 5 cm length, showing the origin of the first medullary bundle from the ventral part of the stele. The separation of this first segment of the internal system breaks the ventral bundle (*v*) into two smaller bundles (v_1 and v_2). × 14. Fig. 12. T.s. of a rhizome (1st medullary bundle stage) at the origin of a short shoot. × 14. Figs. 13-15. A series of closely successive t.s. through a rhizome at about 20 cm length, showing the origin of the second medullary bundle. Note its origin as two separate strands (*a* and *b*) from each of two ventral bundles, the secondary fusion with the 1st medullary bundle (Figs. 14 and 15) and the ultimate fusion with one another to form a single strand (Fig. 15). × 14.

The single medullary bundle stage is very long and stable. A wild sporeling found at Plymouth, Massachusetts, with rhizomes 3 cm long, had not progressed any further, and many rhizomes over a foot in length, grown in the greenhouse, were still at this stage. After about 20 cm or more of rhizome have been produced, one or two of the lower peripheral bundles expand a second time and bud off another internal bundle, or more usually two small bundles, which later fuse. Jeffrey (1902) states that this second medullary strand may arise either as just described, from involution of the lower peripheral strands, or from dichotomy of the already existing axial bundle. The latter has not been observed during the course of this study, although immediately after the second bundle is formed, it is displaced dorsally and joins temporarily with the first. When this bundle is initially formed as two separate strands (as often is the case), these may join as they move upward or may remain separate until after they join temporarily with the first internal strand. In either case, they ultimately anastomose to become a single, elongate bundle (Figs. 12-15). The secondary proximity of the two axial bundles may be what Jeffrey interpreted as a method of their origin. In adult material, strands derived from the medullary bundles, are often seen separated in transverse section. These connect the medullary and peripheral systems between the nodes.

In several sporelings examined, precocious establishment of second medullary strands was observed, at levels between six and fifteen centimeter of rhizome length. A strand arises from the lower part of the stele in the normal manner, moves centrally, runs for about a millimeter or more, and then dies out or joins with the first bundle and is lost. Some specimens revealed as many as two or three such abortive formations before a stable strand was finally established.

Once the second central strand is established, the adult stage has been reached. It must be emphasized once more that all the bundles seen in the adult stem arise from the two strands of the original rhizome stele by "budding" without evidence of relation to leaves or branches. In addition, all the bundles in the stem, including those in the center, anastomose with one another irregularly throughout the internode, making a highly coherent, albeit complicated system. The inner bundles connect with the peripheral ones sporadically in the internodes and regularly at the short shoot origins, where all contribute to the branch traces. The roots connect solely with the peripheral bundles. Table 1 summarizes the data of this ontogenetic sequence and contains a series of stereodiagrams showing the random bundle anastomoses and dichotomies which give the vascular system a meshwork appearance.

3. Vascularization of Short Shoots and Leaves — No treatment of the vascular anatomy of bracken would be complete without a section devoted to the long shoot - short shoot and short shoot-leaf vascularizations. In the adult plant, this has been worked out by Bayer (1903), Tansley & Lulham (1904) and Webster (1957). These authors agree that the short shoot has essentially the same vascular anatomy as the long shoot and at the juncture of the two, the medullary and peripheral bundles of the side axes become neatly continuous with comparably situated bundles in the main rhizome. The base of the bracken stipe has a peculiar appearance in cross-section (*see* Bayer, Webster, or Table 1); there is a sclerenchymatous strip centrally, resembling an inverted letter T, and from four to nine vascular bundles surrounding it, the two on either side of the shaft of the T being typically the largest. The junction of leaf and short shoot reveals that the axial or medullary bundles of the short shoot vascular system are the source of these two large foliar strands, while the smaller bundles are derived from minor, peripheral strands. Bayer has given an excellent series of illustrations showing the exact nature of all these connections, and although there is minor variation among fronds in number of bundles and their attachment and anastomoses through the stipe, the basic plan remains constant, viz. both the medullary and peripheral conducting systems contribute to the short shoot and leaf vascular supply.

Stages in the establishment of this complex foliar and short shoot pattern of vascularization may be observed in the sporeling (*see* Table 1 for illustrations). The first two or three sporeling leaves have a very simple petiolar vascularization,— merely a small, oval mass of tracheids (with no detectable phloem) and an enclosing, dark-staining endodermal layer. There is no disturbance of the protostelic stem at their insertion, merely an adjoining to the periphery of the cylinder. Leaves four to eight progress gradually from flattish or slightly crescent-shaped petiolar bundles to strongly recurved strands resembling the letter C with its open ends reflexed. This single strand type persists until the siphonostelic structure of the rhizomes begins to break up and the first short shoots appear. Then the strap-shaped leaf supply divides into two or four strands, one joining the axial bundle and the others connecting with the peripheral bundles of the shoot cylinder. Concurrent with the attainment of adult stelar pattern comes the ultimate transition to the adult petiolar configuration, complete with increased number of bundles, sclerenchyma in the form of an inverted T, and adult pattern of vascular attachment to the short shoot vascular system.

Apart from this gradual metamorphosis toward adult configuration, sporeling leaf-short shoot attachments are transitional in quite another way. Up to and even beyond the attainment of shoot vascular maturity, the first frond produced on every short shoot retains at least one direct vascular attachment to the long shoot. As can be seen in cleared material (Figs. 16a and 16b, 17 and 18), most of the

FIGS. 16-18 — Fig. 16a. A cleared *Pteridium* sporeling showing growth habit, short shoot and leaf vascularizations. × 1·4. Fig. 16b. The boxed portion of Fig. 16a enlarged to show the short shoot vascularization and the vascular strands running directly from leaf to long shoot (arrows). This section precedes the origin of the medullary system. × 16. Figs. 17, 18. Cleared rhizome sections showing short shoot and leaf vascularizations at the first medullary bundle stage (Fig. 17) and the second medullary bundle stage (Fig. 18). In both, the direct foliar to rhizome connections are clear (arrows). × 16.

vascular strands which supply the leaf are derived from the short shoot, but at the angle between leaf base and long shoot, one or two conducting strands develop which connect the leaf directly with the vascular system of the main rhizome (usually to one of the upper, peripheral strands). The short shoot apex arises by an unequal separation of the long shoot apex into two portions as shown by Webster (1957). According to her, the first leaf produced after the establishment of the short shoot is produced on the side axis on the side between it and the long shoot. Considering the initial proximity of all these structures at the apex, it is not difficult to visualize the differentiation of direct vascular connections between leaf and both long and short shoots.

The succeeding leaves borne by the short shoots generally derive their entire vascular supply from the parent short shoot. However, in many cases of greenhouse-raised sporelings where two leaves were produced by the short shoot in rapid succession, even the second leaf had a single bundle running directly down to and connecting with the long shoot. Webster (1957) reports that adult material obtained from the field shows no sign of these direct long shoot leaf connections so universal in the sporeling. It is not at all clear when these connections cease to be produced, because they were present even in typically adult plants raised from sporelings in the greenhouse, with main rhizomes more than a meter long.

Discussion

In view of the importance of the initial appearance of pith to the whole subject of fern ontogeny, some discussion of the phenomenon might be à propos here. The single layered fern apical meristem consists typically in Leptosporangiate ferns of a large, central *apical cell* flanked by its elongate, longitudinal derivatives, the *prismatic cells*. Beneath this, and derived from cells cut off from the bottom of the meristematic cells is a disc or cap of potential vascular tissue — the *prestelar zone*. If all the basipetal progeny of this region elongate and mature into conducting elements, the prestelar zone will be continuous below with a protostele. However, some of the cells in the center of this embryonic cap may undergo frequent divisions perpendicular to the shoot axis, and form a pith or medulla consisting of rows of parenchyma cells and occupying the center of the vascular cylinder. This medullation leads to the condition described as siphonostely. The important point here is that regardless of the nature of the mature stele, whether solid, tubular, or even more complex, the prestelar zone in the apical region is probably all potentially vascular, i.e. every cell produced by it is theoretically capable, under the proper conditions, of developing into a conducting element. However, this prestelar zone is not necessarily uniform in appearance (as Wardlaw, 1945, has suggested for some ferns), i.e. the central region may differ from the periphery. This has been shown for *Osmunda* by Steeves (1951), and will be discussed for *Pteridium* in a subsequent paper. The investigation of the change in destiny of potential vascular tissue, which accompanies stelar expansion and leaf origin, remains an important morphogenetic problem.

The possible role of the leaves in this origin of pith is not yet clear. In *Pteridium*, the pith typically arises between 160 and 200 μ below the level of the first leaf gap. Transverse sections of young sporelings have invariably revealed small, parenchymatous nests in the center of the stele at this level. However, the distance below the first leaf gap represents only a few cell layers, so that this evidence is not sufficient to rule out a possible relation between leaf development and the medullation of the stele. Certainly the increasing size and photosynthetic (laminar) area of the leaves plays an important synthetic role in contributing to the nutritional status of the shoot apex as a whole. There is the possibility that some critical chemical level must be reached before medullation is triggered, perhaps by a hormone, and perhaps a hormone largely produced in and diffused from the leaves. Thus, although the pith is distinctly cauline stelar tissue, the factor or factors which influence its inception

could be foliar in origin. Therefore, while it may be said that medullation is an internal transformation within the stele, the mechanisms involved in the apical regions remain, for the present, quite obscure.

The origin of the medullary vascular system represents a critical step in the transition from sporeling to adult anatomy. In some individuals, short shoots are established before this stage, and certainly by the time the first medullary bundle is formed, every sporeling raised on soil during this work had made the transition to leaf production on side axes. Viewed developmentally, the appearance of vascular tissue, in what was previously pith, involves a second "change of destiny" of embryonic cells in the apical region. (The first such change involved the xylem-pith transition at the onset of the siphonostelic stage.) In sporelings between the siphonostele and initial medullary bundle, the peripheral areas of the prestelar zone give rise below to the heterogeneous components of the vascular "bundles," while the central section is continuous with the large, highly vacuolate pith tissue. When the first medullary bundle is formed, the potential vascular zone in the region of the lower peripheral bundle becomes more extensive at the expense of some incipient pith tissue. Eventually, the prestelar zone produces this additional vascular tissue apart from its parent area, and the first medullary strand is differentiated from a central section of the prestelar layer, separated from the rest of the vascular system by pith and sclerenchyma.

The vestige of a vascular attachment between the leaf and the long shoot of advanced sporelings recalls sharply the earlier ontogenetic stages of the plant. A close look at sporeling ontogeny has already shown that leaves are produced directly on the rhizomes when these are first formed by apical dichotomy of a vertical axis; and, in fact, three plants were observed to revert to this direct leaf production even after the short shoot habit had been established. Two of the cases followed equal rhizome dichotomies, the small daughter rhizomes producing one leaf each directly before going over to short shoot formation again. The third case involved a rapidly growing rhizome which reached the end of the flat in which it was planted. As the stem apex was deflected, it produced a leaf directly. The plasticity of the sporeling shoot apex is revealed quite amply by this, for, in spite of important differences between leaf primordium inception and branch production, the long shoot apex is apparently able to shift from one to the other under suitable conditions. In culture, the long shoots revert to leaf production directly as the medium approaches exhaustion. In view of current morphogenetic interest in branches, buds, and leaves (*see* Wardlaw, 1952, for a review) and the interesting long shoot-short shoot morphology in *Pteridium*, there is obviously a great deal to be gleaned from an experimental study of the bracken sporeling.

Webster (1957), in speculating about the evolution of the *Pteridium* stele, suggests that phylogenetically the long shoot was strongly influenced by the large leaves which it once bore directly. Now, however, it is frondless and vascular development is entirely under cauline influence. The presence of leaves on the sporeling long shoot and the transient vascular connections between the rhizome and these leaves increase the credibility of Webster's hypothesis and once more point up the potential of this plant for morphogenetic investigation. Sporelings of *Pteridium* are particularly valuable for experimental studies on both external morphology and internal anatomy since the changes are so regular and well defined. Some studies along these lines will be reported in a subsequent paper.

In conclusion, two additional steps may be added to the series of stelar ontogenetic changes outlined in the introduction to this report:

6. "Perforation" of the stele, so that more gaps are seen in cross-section than can be directly traced to leaf or branch gaps (*Pteridium*, *Phlebodium*).

7. Establishment of concentric vascular cylinders by budding of vascular tissue from the inner surface of the outer or original bundles (*Matonia*, *Pteridium*, *Marattia*).

Summary

The stelar ontogeny of *Pteridium* has been traced with some reference to its developmental significance. The sporeling vascular system passes through six well defined stages:

1. The *Simple Protostele* — a short stage at the base of the young plant (up to the two-leaf stage). About a dozen tracheids can be seen in cross-section. The leaf traces are single, oval-shaped xylem masses and are joined simply to the periphery of the stele.

2. The *Ectophloic Siphonostele* — at the level of leaf 3 or 4 a small nest of parenchyma cells appears in the center of the stele. This medullation is discussed historically and developmentally. Incipient phloem tissue surrounds the xylem externally and the endodermis takes on the adult black staining, indicating the presence of phenolic compounds. Leaf gaps appear at about the four-leaf stage.

3. The *Two-strand Stage* — the leaf gaps of leaves 6 through 8 overlap and the stele is broken into two vascular bundles. Internal phloem and endodermis are distinct and the leaf traces are crescent-shaped. The distribution of the vascular tissue at forking is dealt with in some detail.

4. Initial *Perforation* — as the rhizomes level out and grow horizontally in the soil, several small, vascular bundles are separated from the flanks of the lower strand. The stele consists of a ring of peripheral meristeles most of which have no direct connection to leaves or branches and are, therefore, said to be derived by stelar perforation. Short shoots are now produced regularly, and on them the leaves are borne.

5. The *First Medullary Bundle Stage* — after 6 cm or more of rhizome are formed, the first medullary bundle is separated from the inner surface of the lower, peripheral bundle. This is discussed morphogenetically.

6. The *Second Medullary Bundle Stage* — the adult condition; this second internal strand arises similarly as an involution from the lower peripheral system.

Direct vascular connections between leaf and long shoot are discussed in connection with nodal anatomy, as well as the generally transitional nature of the sporeling shoot system. A review of stelar terminology is presented at the beginning of the paper.

For his valuable suggestions during the progress of this work as well as his painstaking perusal of the manuscript, my sincerest thanks are extended to Professor Taylor A. Steeves. My gratitude goes, too, to Professor Ralph H. Wetmore for his help with the background of the stelar theory and his advice on the writing of this report. This work was done with the aid of generous scholarships from Radcliffe College and a Mary Andersen Fellowship from the American Association of University Women. This paper is based upon part of a thesis submitted to the Department of Biology of Radcliffe College in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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REVIEW

RUSSELL, NORMAN H. 1958. "An Introduction to the Plant Kingdom." C.V. Mosby Company, U.S.A. Pp. 353.

THIS book has been written for those pursuing a one-semester elementary botany course on the "plant kingdom". In Russell's opinion none of the previous textbooks on Botany exactly answers this requirement.

The text is divided into 28 chapters. The first entitled "The Plant Kingdom" deals with three general topics: Names of Plants, Classification of the Plant Kingdom and Organic Evolution. Chapters 2-27 embrace the algae, fungi, mosses, liverworts, lower vascular plants and flowering plants. The last chapter bears the heading "The Evolution of Plants — A Summary". At the end follow the Glossary and the Index. Topics dealing with plant physiology, ecology and genetics are outside the scope of the book and have, therefore, received little or no attention.

Throughout the text the "type" method is used. One is greatly impressed by the stress laid on the evolutionary aspect and the economic importance of the various plant groups. The descriptions are brief but lucid and at the same time interesting. The selected references given at the end of each chapter enhance the value of the book.

Of the illustrations, the photographs are exceedingly good, but the line drawings are insufficient and many are quite amateurish. To cite a few examples: the account of the life history of *Puccinia graminis* has not been illustrated at all; there are photographs for the external morphology of lichens, but nothing to explain their internal structure; and in the account of *Pinus* there are no figures of the development of male gametophyte, ovule and embryo. Fig. 147 of a lily ovule shows the two integuments fused except at their tip; in Fig. 149 of the seed of *Capsella* the plumule has been labelled as the epicotyl.

In the Preface it is claimed that the Glossary is "extensive" but this does not really appear to be so. While terms like annual, perennial, gynoecium, endarch, exarch, cambium, procambium, cortex, bark, monopodial have been listed, other related ones like biennial, androecium collateral, bicollateral, cork cambium, secondary cortex, periderm and sympodial find no mention. Some of the definitions are also not quite exact. For instance, the one for *nucellus* reads: "The nutritive tissue surrounding the egg in the ovule; the modified megasporangium."

The paper, printing and get-up of the book are good, but there is no mention of the price.

R. N. CHOPRA